VOLUME 64
NUMBER 10 / NUMÉRO 10
OCTOBER / OCTOBRE
2007

Canadian Journal of FISHERIES AND AQUATIC SCIENCES

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Journal canadien des SCIENCES HALIEUTIQUES ET AQUATIQUES

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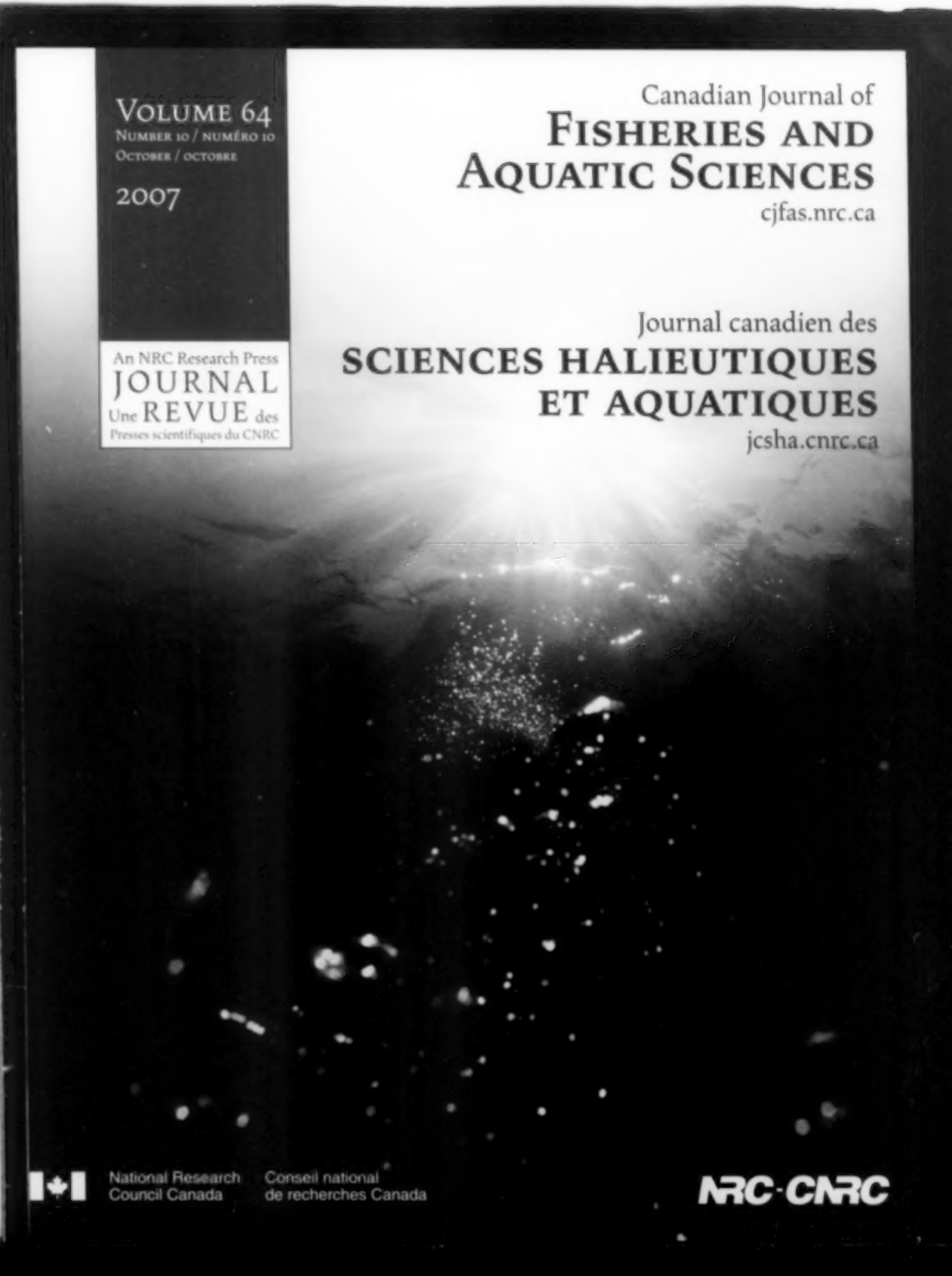

National Research Council Canada
Conseil national de recherches Canada
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ISSN 0706-652X / CJFSDX 64(10) 1291–1468 (2007)

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Volume 64, Number 10, October 2007

Volume 64, numéro 10, octobre 2007

Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

Published by the National Research Council of Canada

Publié par le Conseil national de recherches du Canada

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Ecological correlates of fish reproductive activity in floodplain rivers: a life-history-based approach

Steven C. Zeug and Kirk O. Winemiller

Abstract: Reproductive activity of seven species representing three divergent life history strategies was monitored monthly for 2 years in channel and floodplain habitats of the Brazos River, Texas, USA, to evaluate associations between reproductive activity and biotic and abiotic factors predicted by conceptual models to influence reproduction. An information-theoretic approach was used to select best approximating models for each species, and model-averaged estimates of regression coefficients were calculated. Model selection indicated that monthly flow based on the 30-year hydrograph and temperature was strongly supported as factors associated with reproductive activity of all three life history strategies. The timing of reproduction in relation to the long-term hydrograph was related to life history traits. Reproductive activity of species with large adult size and high fecundity was greatest in spring just prior to increasing flows, whereas species with small adult size and extended breeding seasons exhibited greater activity in late spring and summer when mean flow was greatest. Nest-building species with parental care were more abundant in off-channel habitats where floods were less common. Instream flow management would benefit from consideration of flow and habitat requirements needed to support the diverse life history strategies displayed by fishes in river–floodplain systems.

Résumé : Nous avons suivi à chaque mois pendant 2 ans l'activité reproductive de sept espèces représentant trois stratégies de cycle biologique distinctes dans les habitats du chenal et de la plaine d'inondation de la Brazos, Texas, É.U., afin d'évaluer les associations entre l'activité reproductive et les facteurs biotiques et abiotiques qui, d'après certains modèles conceptuels, l'influencent. Nous utilisons une approche basée sur la théorie de l'information pour sélectionner les modèles qui s'ajustent le mieux à chaque espèce et nous calculons à l'aide des modèles les estimations moyennes des coefficients de régression. La sélection des modèles indique que le débit mensuel basé sur la courbe de 30 ans et la température sont les facteurs le plus fortement retenus pour expliquer l'activité reproductive dans les trois stratégies de cycle biologique. La détermination du moment de la reproduction en fonction de la courbe de débit à long terme dépend des caractéristiques du cycle biologique. Les espèces avec adultes de grande taille et à forte fécondité ont une activité reproductive maximale au printemps juste avant l'accroissement des débits, alors que les espèces avec des adultes de petite taille et une saison de fraye étendue montrent plus d'activité reproductive à la fin du printemps et en été quand le débit moyen est maximal. Les espèces qui construisent des nids et qui prennent soin de leurs petits sont plus abondantes dans les habitats hors du chenal où les crues sont moins fréquentes. La gestion des débits dans les cours d'eau pourrait être améliorée si on tenait compte des exigences de débit et d'habitat requises pour le maintien des diverses stratégies de cycle biologique possédées par les poissons des systèmes de rivières et de leurs plaines d'inondation.

[Traduit par la Rédaction]

Introduction

The critical role of hydrology to ecological dynamics in river–floodplain systems is well recognized (Poff et al. 1997; Bunn and Arthington 2002). Alteration of natural flow regimes due to dam and levee construction, water extraction, and channel modification has been associated with myriad impacts on aquatic fauna (Moyle and Light 1996; Tockner and Stanford 2002; Agostinho et al. 2004). In response to these impacts, instream flow regimes have been developed to restore the ecological integrity of modified systems (Jowett 1997; Richter et al. 1997). Most of these strategies focus on minimum flows, flow variability, or habitat availability and may produce conflicting assessments depending on the method used (Jowett 1997). Ecological data relevant to restoration of modified river systems is often lacking (Naiman et al. 1995; Richter et al. 1997), and conceptual models of ecological function can be useful to guide restoration strategies (Trexler 1995). Large rivers exhibit a wide range of flow dynamics, and conceptual models should be evaluated

Received 21 November 2006. Accepted 24 May 2007. Published on the NRC Research Press Web site at cjfas.nrc.ca on 21 September 2007.
J19673

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 doi:10.1139/F07-094 © 2007 NRC Canada

before application to river management strategies (Thorp et al. 2006).

For over a decade, the flood pulse concept (FPC; Junk et al. 1989) has been viewed as the best approximation of ecological function in large lowland rivers. The FPC emphasizes the importance of pulsed lateral connections to aquatic productivity and maintenance of biotic diversity. The timing of reproduction by fishes is predicted to coincide with flood pulses that allow placement of offspring in floodplain habitats where they can exploit the high productivity associated with the aquatic–terrestrial transition zone. In temperate regions, optimal conditions for reproduction are predicted to occur when flooding coincides with appropriate temperatures, and lower recruitment is predicted when these conditions are not met (Bayley 1991). Humphries et al. (1999) proposed the low-flow recruitment hypothesis (LFR) for rivers in which flood dynamics are unpredictable or flood pulses do not coincide with rising temperature. This model recognizes the importance of lateral connectivity to ecological dynamics, but proposes that fishes time reproduction to coincide with predictable low flows when temperatures are warm and food resources are concentrated.

Floodplain rivers support fish species with a diversity of life history strategies (Humphries et al. 1999; Winemiller et al. 2000; King et al. 2003). Environmental conditions differentially affect reproduction and recruitment of species with different life history strategies (Magalhaes et al. 2003; Olden et al. 2006), and model predictions of reproduction (e.g., FPC) may only apply to a subset of species with a particular suite of traits. Flow management strategies that benefit one life history strategy may be detrimental to species that rely on alternate flow conditions (Sparks 1995; Scheerer 2002; Welcomme et al. 2006) or off-channel habitats that frequently are overlooked in instream flow management. Integration of conceptual models of reproduction with fish life history strategies can provide valuable information regarding flow management strategies necessary to support the diversity of fishes that occur in large rivers.

Our goals for this study were to identify biotic and abiotic factors associated with reproductive activity of fishes with divergent life history strategies and compare patterns of fish reproductive activity and adult abundance within channel and floodplain habitats. We predicted that combinations of biotic and abiotic factors related to reproductive activity would be strongly associated with life history strategy. We also predicted that the value of habitats for reproduction by fishes with different life history strategies would be associated with hydrologic connections among aquatic habitats.

Materials and methods

Study system

The mainstem Brazos River flows 1485 km from its origin near the Texas – New Mexico border to the Gulf of Mexico near Freeport, Texas, USA. The current study was conducted on the Middle Brazos between 30°25′N and 30°37′N. In this region, the Brazos is a meandering, low-gradient river bordered by forested and agricultural lands and drains a 76 361 km^2 catchment. Oxbow lakes are common on the Middle Brazos floodplain, and hydrologic connections between oxbows and the channel are relatively unpredictable both within and among years (Winemiller et al. 2000; Zeug et al. 2005). The Middle Brazos is less modified by dams and levees than other North American floodplain rivers, and although flow is partially regulated by dams in and above the city of Waco, Texas, current flow dynamics are relatively similar to predam flows.

A previous survey of 10 Brazos oxbow lakes by Winemiller et al. (2000) found that oxbows with similar geomorphology had similar fish assemblages. Two of these oxbow lakes with different connection frequencies and a 7 km reach of the Brazos River were surveyed monthly from June 2003 to May 2005. One oxbow (OXFREQ) connects to the active channel relatively frequently at moderate levels of Brazos River discharge, and one oxbow (OXRARE) connects relatively rarely at high river discharge. The two oxbows were selected to span the range of oxbow connection frequencies based on the previous survey. Both oxbows were located on cattle ranches; however, both lakes retained an unaltered riparian zone dominated by willow (*Salix* sp.). Oxbow substrates were composed primarily of mud and clay covered by leaf litter. Large woody debris was common in both habitats, and aquatic macrophytes were rare. Overbank flooding is the primary source of water for both oxbow lakes, and extended periods of isolation result in oxbow desiccation. Mean depth for OXFREQ and OXRARE during the study was 1.2 and 1.9 m, respectively. The surveyed reach of the Brazos River was selected based on boat access and proximity to the uppermost oxbow lake. Flows required for oxbow–river connections were estimated by surveys conducted by the Texas Water Development Board (TWDB) and were calibrated to a United States Geological Survey (USGS) gauge located near the Brazos River survey reach.

Abiotic predictor variables

Two variables representing flow regime were estimated to reflect different temporal scales to which species may respond. Floodplain connectivity during the current study period was measured as the days of habitat isolation calculated from oxbow connectivity estimates provided by the TWDB; mean daily flow was provided from USGS gauge 08108700. Given that the timing of reproduction could represent adaptation to the long-term hydrograph, the mean flow for each month of the year based on the previous 30 years of data was also included as a predictor variable. Temperature and dissolved oxygen were measured during each survey using a YSI 85 handheld meter. Mean photoperiod in Brazos County during each month was obtained from the United States Naval Observatory, Astronomical Applications Department.

Biotic predictor variables

Zooplankton were collected using a 10 L Schindler trap with 80 µm mesh in the cod end. Individuals were identified as rotifers, cladocerans, or copepods, and densities were determined from two 1 mL subsamples using a Sedgwick–Rafter counting cell. Predator abundance was estimated as the combined gillnet catch per unit effort (methodology described below) of alligator gar (*Atractosteus spatula*), spotted gar (*Lepisosteus oculatus*), longnose gar (*Lepisosteus osseus*), blue catfish (*Ictalurus furcatus*), channel catfish (*Ictalurus punctatus*), largemouth bass (*Micropterus*

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salmoides), spotted bass (*Micropterus punctulatus*), and white crappie (*Pomoxis annularis*).

Fish collection

Large-bodied fish (>100 mm standard length, SL) were collected with two experimental gill nets that consisted of three panels measuring 16.5 × 2 m, with 25.4, 76, and 51 mm bar mesh. Gill nets were set between approximately 1700 and 0700 the next day. The duration in hours of each set was recorded for calculation of species catch per unit effort (CPUE). Small-bodied fish (<100 mm SL) were collected using a 10 m × 2 m bag seine with 6.4 mm mesh in the wings and 3.2 mm mesh in the bag. A series of seine hauls was made perpendicular to shore along unique transects within the habitat until no new species were collected. The total number of metres traveled per survey was estimated, and CPUE was calculated as species number·m^{-1}. Electrofishing was used to supplement samples of species associated with woody debris (bluegill, white crappie) that were not as easily captured with seines and gill nets. Collections were not made in the Brazos River during certain months because of high flows, and oxbows were not sampled during November 2004 because of extensive flooding. All fishes were euthanized by immersion in tricaine methanesulfonate (MS-222). Large fish were placed on ice, transported to the laboratory, and stored frozen. Small fish were fixed in a 10% formalin solution and stored in 70% ethanol.

Based on their positions within the triangular life history continuum proposed by Winemiller and Rose (1992), seven species were selected for estimation of reproductive activity in relation to biotic and abiotic characteristics. Among Brazos River fishes, the western mosquitofish (*Gambusia affinis*) and red shiner (*Cyprinella lutrensis*) represent the opportunistic strategy; bluegill (*Lepomis macrochirus*) and white crappie represent the equilibrium strategy; and gizzard shad (*Dorosoma cepedianum*), spotted gar, and longnose gar represent the periodic strategy. Opportunistic strategists are characterized by small adult size, short generation time, high reproductive effort, and extended breeding seasons. Species with this strategy can quickly colonize disturbed or newly available habitats. Equilibrium strategists have characteristics (high juvenile survivorship, parental care, and large eggs) inferred to be adaptive in habitats where resources are limited and (or) density dependence is strong. Periodic strategists are characterized by delayed maturation, large adult size, high fecundity, and contracted breeding seasons. This strategy is predicted to perform well when resources for larvae are patchy in space or time (Winemiller and Rose 1993), and reproduction is synchronized with favorable periods that are relatively predictable between years. Two gar species were included here, because there is strong habitat partitioning between these species. Longnose gar are more abundant in the river channel, and spotted gar are more abundant in oxbows (Winemiller et al. 2000; Zeug et al. 2005).

To validate that species used for analysis represented the three endpoint strategies in the Winemiller–Rose life history model, correspondence analysis (CA) was performed on six life history characteristics estimated for each species (Fig. 1). Characteristics included in CA were minimum length at maturity (L_{min}), maximum length (L_{max}), mean fecundity, length of the reproductive period, mean egg size, and the presence or absence of parental care. Minimum length at maturity was defined as the smallest female observed with ripe gonads, and maximum length was defined as the largest female collected. Length of the reproductive period was estimated as the number of months when ripe females were collected. Females were classified as ripe based on the gonadosomatic index (GSI) and presence of mature oocytes in ovaries. Mature oocytes were those as big as or bigger than the greatest modal egg size observed during the reproductive period of each species. Information regarding the presence or absence of parental care was obtained from literature.

Sample processing

Reproductive activity was estimated using the GSI for females ((gonad mass/body mass) × 100) (Figs. 2, 3). Individuals were measured to the nearest millimetre SL and weighed to the nearest 0.01 g. Ovaries were removed, blotted dry, and weighed to the nearest 0.001 g on an analytical balance. Ovaries were stored in a 10% formalin solution buffered with sodium phosphate for later fecundity and egg size estimation. For most species, all individuals were processed. Large numbers of mosquitofish and red shiner were collected in monthly surveys (>100 individuals); for these species, individuals were placed into 5 mm size classes, and three individuals from each size class were processed.

Red shiner and mosquitofish fecundity was estimated by direct count. Red shiner ovaries contained multiple batches of eggs based on size frequency distributions, and only the largest size class was used for fecundity estimation (batch fecundity). For all other species, a subsample of the anterior portion of the ovary was removed and fecundity calculated as

$$F_{total} = (OW_{total} \times E_{sub})/OW_{sub}$$

where OW_{total} is the total ovary weight, E_{sub} is the number of eggs in the subsample, and OW_{sub} is the weight of the subsample. Mean egg size was estimated by measuring the largest egg in the ovary or subsample and averaging values for all individuals of a species in the sample.

To determine if GSI was a sufficient predictor of reproductive activity, linear regressions were performed to test the null hypothesis of no difference between GSI and modal egg size for each species. For mosquitofish, embryo developmental stage was substituted for egg size (Haynes 1995). Additionally, regressions were performed to determine if body size (SL) within species was related to GSI. The relationship between GSI and egg size or developmental stage was positive and significant for all seven species, indicating that GSI is an appropriate response variable (red shiner: R^2 = 0.48, $P < 0.001$; mosquitofish: $R^2 = 0.16$, $P < 0.001$; gizzard shad: $R^2 = 0.50$, $P < 0.001$; spotted gar: $R^2 = 0.10$, $P = 0.03$; longnose gar: $R^2 = 0.48$, $P < 0.001$; white crappie: R^2 = 0.45, $P < 0.001$; bluegill: $R^2 = 0.21$, $P < 0.001$). Additionally, peaks in juvenile abundance closely followed periods of high GSI (S.C. Zeug, unpublished data). The relationship between SL and GSI was significant for mosquitofish ($R^2 = 0.10$, $P < 0.001$) and spotted gar (R^2 = 0.11, $P = 0.03$). For these species, residuals of the relationship between SL and GSI were used as the response variable in place of raw GSI.

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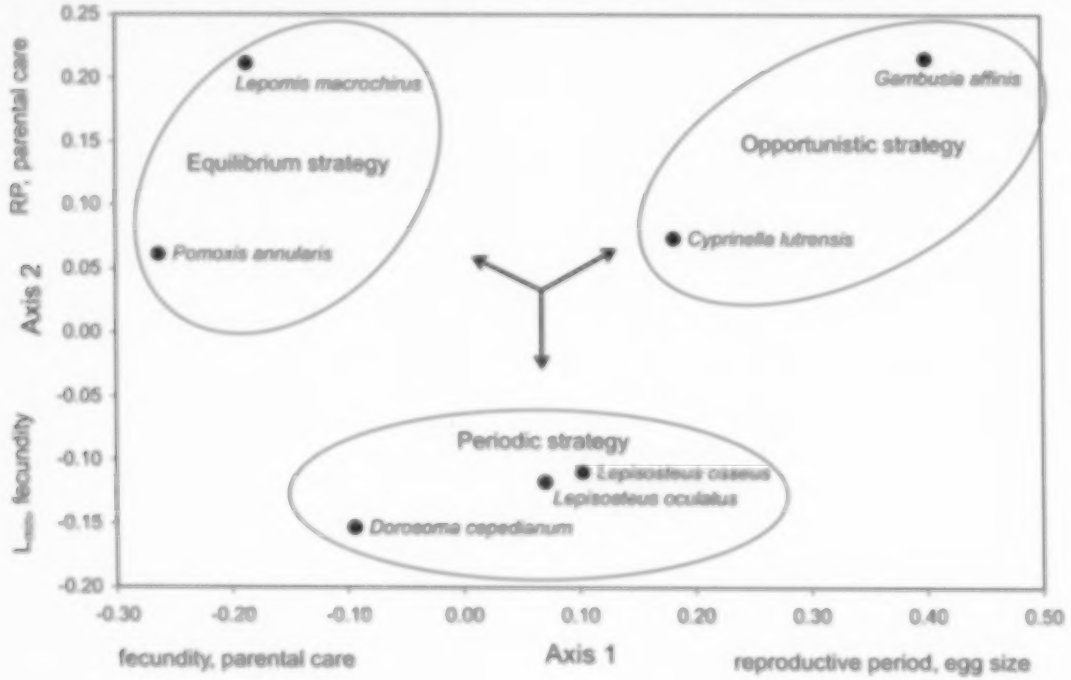
Fig. 1. Ordination of species scores from correspondence analysis based on the six estimated life history characteristics. Axis 1 modeled 59.6% of the total variation; axis 2 modeled 25.5% of variation. Axis 1 variable loadings: minimum length at maturity (L_{min}) = 0.45, maximum length (L_{max}) = 0.36, reproductive period (RP) = 1.41, fecundity = –0.82, parental care = –6.19, egg size = 2.05. Axis 2 variable loadings: L_{min} = –0.27, L_{max} = –0.03, RP = 2.40, fecundity = –0.48, parental care = 7.07, egg size = 0.29.



Model construction and selection

Model sets were constructed for each species based on biotic and abiotic factors predicted by conceptual models (FPC, LFR) to influence fish reproductive activity. Both conceptual models predict associations among reproductive activity, flood dynamics, temperature, and larval food resources. The density of predators of early life stages also can be influenced by water level (Copp 1992) and was included as a predictor variable. Measured variables considered for inclusion in models of reproductive activity included mean monthly flow for the previous 30 years, days of floodplain isolation, temperature, photoperiod, rotifer density, copepod density, cladoceran density, and predator abundance.

Correlation analysis was performed on independent variables to identify potential sources of multicolinearity. Temperature was significantly and positively correlated with rotifer density and photoperiod. Temperature was retained, and rotifer density and photoperiod were excluded from the analysis. Temperature was retained because of its predicted importance in conceptual models. Cladoceran and copepod densities were combined into the variable "microcrustacean density". Tests for normality were performed on dependent variables (GSI or residuals) prior to model construction. When the assumption of normality was not met, data were log-transformed as $\log_{10}(x + 1)$. Surveys in the Brazos River produced few crappie and bluegill; thus, these species' models included samples from oxbows only. Additionally, the longnose gar model was calculated using samples exclusively from the Brazos River.

Independent variables used in models included 30-year hydrograph, isolation days, microcrustacean density, temperature, and predator abundance. To insure that models reflected biological reality as closely as possible, some variable combinations were excluded from consideration. For example, predator abundance is predicted to be greater during low-water periods and lower during high-water periods when aquatic habitats are expanded (Copp 1992). The variable isolation days reflected this change in water level during the study, whereas the 30-year hydrograph was not linked to conditions during the study when predator abundance was estimated. Therefore, predator abundance was considered in conjunction with isolation days but not with the 30-year hydrograph. Twenty-one candidate models were evaluated. Generalized linear models were constructed for mosquitofish, red shiner, longnose gar, spotted gar, and bluegill, whereas autoregressive models were constructed for shad and white crappie because of first-order autocorrelation in model residuals. Both modeling techniques utilized maximum likelihood to estimate model parameters. All models were constructed using SAS (Version 9.1.3, SAS Institute Inc., Cary, North Carolina).

Best approximating models were selected using an information-theoretic approach. Because it allows the evaluation of evidence in data for multiple working hypotheses, this methodology is superior to traditional hypothesis testing

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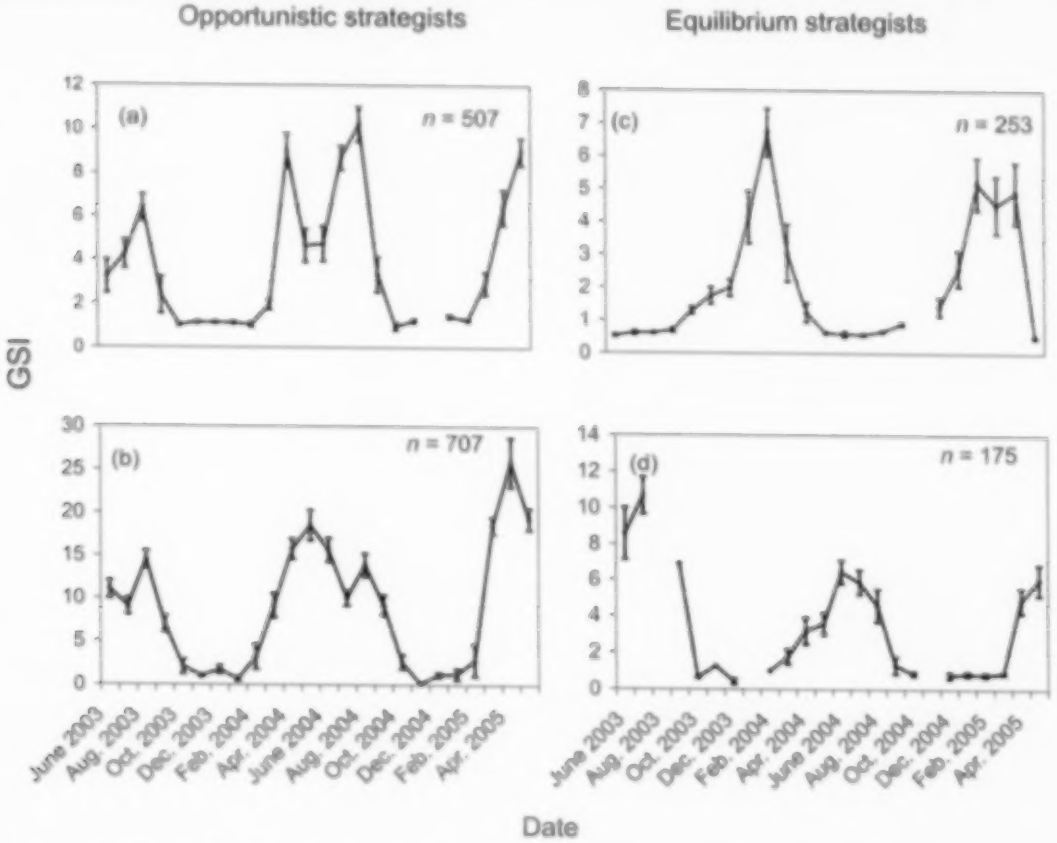
Fig. 2. Plot of mean gonadosomatic index (GSI) values over the 2-year study period for the two equilibrium and opportunistic strategists. Species in each panel are as follows: (*a*) red shiner (*Cyprinella lutrensis*), (*b*) mosquitofish (*Gambusia affinis*), (*c*) white crappie (*Pomoxis annularis*), and (*d*) bluegill (*Lepomis macrochirus*).



when using observational data (Burnham and Anderson 2002; Hobbs and Hilborn 2006). In the present study, each candidate model was considered a working hypothesis. This methodology has been successfully used to evaluate models for a wide range of ecosystems (Harig and Faush 2002; Sztatecsny et al. 2004; Torgersen and Close 2004).

Akaike's information criterion corrected for small sample size (AIC_c) was used to select best approximating models from the candidate set. Models were ranked using ΔAIC_c values, AIC_c weights (w_i), and evidence ratios. Model weights are interpreted as the probability that the model would be selected as the best model after many repetitions of model selection (Hobbs and Hilborn 2006). Evidence ratios estimate support in the data for two models based on model weights. Ratios were computed as w_i/w_j, where w_i is the model weight for the best model in the candidate set, and w_j is the model weight of the model being considered. Models with $\Delta AIC_c < 2.00$ and evidence ratios < 2.70 were considered competing models. To reduce model selection uncertainty, model-averaged estimates of regression coefficients and unconditional standard errors were calculated from the entire model set. Model averaging can reduce model bias and increase precision of regression coefficient estimates (Burnham and Anderson 2002). Unconditional 95% confidence intervals were used to determine the level of support for regression coefficients. When confidence intervals included zero, the coefficient was assumed to have little support.

Species abundance

CPUE of adults of each species was compared among habitats using generalized estimating equations. Abundance estimates were log-transformed ($\log_{10}(x+1)$) to meet assumptions of the generalized estimating equation procedure. The variable "month" was included as the repeated variable in the model, and an autoregressive correlation structure among months was specified. When significant differences were detected, pairwise tests were conducted. Because the same response variable was used in multiple tests, P values were corrected using the Bonferroni correction ($\alpha_{adjusted} = 0.013$).

Results

Life history classification

CA produced two axes that explained 85.1% of the variation in species life history characteristics (Fig. 1). Three end-point strategies were well differentiated in the CA ordination. Axis one contrasted opportunistic species (red shiner and mosquitofish) that had longer reproductive periods and

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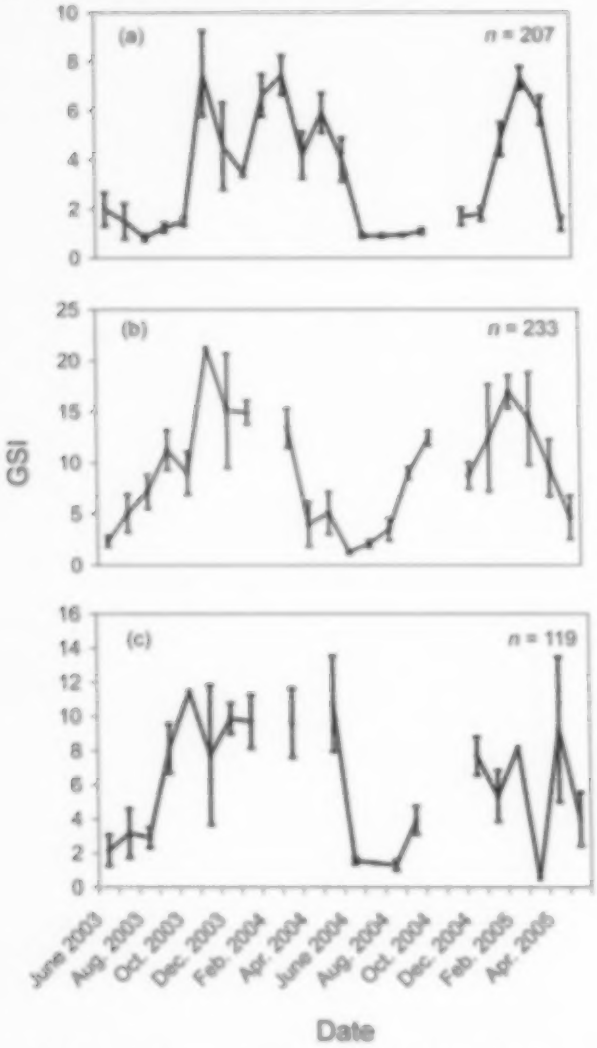

Fig. 3. Plot of mean gonadosomatic index (GSI) values over the 2-year study period for the three periodic strategists. Species in each panel are as follows: (*a*) gizzard shad (*Dorosoma cepedianum*), (*b*) spotted gar (*Lepisosteus oculatus*), and (*c*) longnose gar (*Lepisosteus osseus*).

small body size from equilibrium species (bluegill and crappie) that had greater body size, fecundity, and parental care. Axis two contrasted periodic species (shad, spotted gar, and longnose gar) that had greater fecundity, body size at maturity, and maximum length from equilibrium and opportunistic species that had more developed parental care and longer reproductive periods.

Flood dynamics

Multiple flood events connected oxbow and channel habitats during the 2-year study period (Fig. 4). Flow dynamics did not appear to follow a seasonal pattern, and floods occurred in all four seasons. Nine flood events connected OXFREQ with the river channel for a total of 56 days of connectivity. Four flood events connected OXRARE with the river channel for a total of 10 days of connectivity. The 30-year hydrograph indicated mean flows were greatest in May and June and lowest in August and September.

Opportunistic strategists

The best approximating model for mosquitofish indicated positive associations among GSI, the 30-year hydrograph, and temperature (Table1). The next best model was more than two times less likely (evidence ratio 2.35) and included variables from the best model with the addition of microcrustacean density. Model-averaged parameter estimates and confidence intervals indicated microcrustacean density had little support (Table 2). The best model selected for red shiner had positive associations among GSI, the 30-year hydrograph, and temperature (Table1). Three competing models contained the two variables from the best model as well as combinations of microcrustacean density (negative association), isolation days (positive association), and predator abundance (negative association) (Table 1). Model-averaged estimates indicated that only the 30-year hydrograph, temperature, and predator abundance had support (Table 2).

Periodic strategists

The best gizzard shad model contained positive associations among GSI, the 30-year hydrograph, and microcrustacean density and negative associations among GSI, days of habitat isolation, and predator abundance. The second best model was 2.57 times less likely and consisted of the previous parameters with the addition of temperature (negative association). Model-averaged estimates indicated little support for isolation days and temperature (Table 2). The highest ranked model for spotted gar included negative associations between GSI and temperature (Table 1). The competing model included temperature and the 30-year hydrograph (negative association), and both variables had good support (Table 2). The best longnose gar model indicated negative associations between GSI and temperature. The next best model (evidence ratio = 2.58) included temperature and a positive association with the 30-year hydrograph, although the hydrograph had little support.

Equilibrium strategists

Two competing models were selected for bluegill (Table1). The best model contained temperature and the 30-year hydrograph as predictors, and both associations were positive. The competing model contained the two predictor variables from the best model and a negative association with microcrustacean density; however, only the predictors from the best model had good support (Table 2). The best model selected for white crappie included negative associations with GSI and temperature and positive associations with GSI and microcrustacean density (Table1). The next best crappie model included the variables from the best model and a positive association with the 30-year hydrograph. This model was 3.41 times less likely compared with the best model, and the 30-year hydrograph had little support.

Adult abundance

Significant differences among habitats were detected for all species, with the exception of mosquitofish (Table 3).

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Fig. 4. Daily stream flow hydrograph of the Brazos River during the 2-year study period. The solid horizontal line represents flows required to connect OXFREQ (an oxbow lake that connects to the active channel relatively frequently) with the river channel; the broken line represents flows required to connect OXRARE (an oxbow lake that connects relatively rarely).

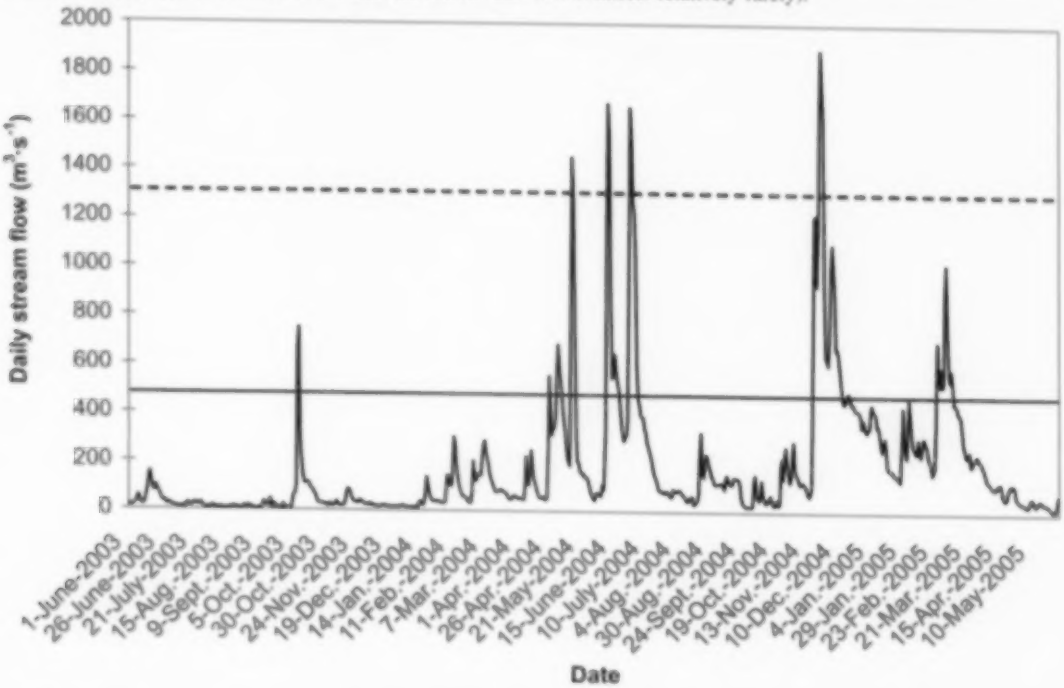

Table 1. Best approximating models for each species based on ΔAIC_c values, model weights, and evidence ratios.

Species	Models	ΔAIC_c	AIC_c weight	Evidence ratio	K
Mosquitofish (*Gambusia affinis*)	β_1, β_2	0.00	0.42	1.00	4
	β_1, β_2, β_3	1.72	0.18	2.35	5
	β_1, β_2	0.00	0.37	1.00	4
Red shiner (*Cyprinella lutrensis*)	β_1, β_2, β_4	1.60	0.17	2.24	5
	β_1, β_2, β_3	1.64	0.16	2.29	5
	β_1, β_2, β_3, β_5	1.90	0.14	2.60	6
Gizzard shad (*Dorosoma cepedianum*)	β_1, β_3, β_4, β_5, β_6	0.00	0.67	1.00	7
	β_1, β_2, β_3, β_4, β_5, β_6	2.57	0.19	3.61	8
Spotted gar (*Lepisosteus oculatus*)	β_2	0.00	0.27	1.00	3
	β_1, β_2	0.11	0.26	1.05	4
Longnose gar (*Lepisosteus osseus*)	β_2	0.00	0.46	1.00	3
	β_1, β_2	2.58	0.13	3.63	4
Bluegill (*Lepomis macrochirus*)	β_1, β_2	0.00	0.42	1.00	4
	β_1, β_2, β_3	1.57	0.19	2.18	5
White crappie (*Pomoxis annularis*)	β_2, β_3, β_6	0.00	0.43	1.00	5
	β_1, β_2, β_4, β_6	2.47	0.13	3.41	6

Note: K, the number of parameters estimated for each model; β_1, 30-year hydrograph; β_2, temperature; β_3, microcrustacean density; β_4, isolation days; β_5, predator abundance; β_6, autocorrelation correction parameter.

Among oxbow lakes, species abundances were similar, with the exception of gizzard shad that were significantly more abundant in OXFREQ than the other two habitats (Table 3). Red shiners were more abundant in the river channel than in oxbows (Fig. 5). Spotted gars were more abundant in oxbows, and longnose gars were more abundant in the river (Fig. 5). Bluegills were more abundant in OXRARE than in the river channel, and white crappies were more abundant in oxbows relative to the river channel (Fig. 5).

Discussion

Model selection indicated that temperature and the 30-year hydrograph had strong support as factors associated

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Table 2. Model-averaged estimates of regression coefficients (unconditional 95% confidence intervals in parentheses).

Species	β_1	β_2	β_3	β_4	β_5	β_6
Mosquitofish	0.55	1.74	–0.03*	–0.02*	0.53	—
	(0.37–0.74)	(1.45–2.02)	(–0.01–0.07)	(–0.08–0.04)	(0.18–0.89)	—
Red shiner	0.49	1.56	–0.03*	0.05*	–0.40	—
	(0.33–0.64)	(1.32–1.79)	(–0.06–0.00)	(0.00–0.10)	(–0.68 – –0.13)	—
Gizzard shad	0.41	–0.03*	0.12	–0.03*	–0.69	–0.32
	(0.28–0.54)	(–0.30–0.24)	(0.09–0.16)	(–0.08–0.03)	(–0.94 – –0.43)	(–0.48 – –0.16)
Spotted gar	–0.31	–1.04	–0.01*	–0.01*	–0.20*	—
	(–0.52 – –0.10)	(–1.37 – –0.71)	(–0.07–0.04)	(–0.08–0.07)	(–0.63–0.23)	—
Longnose gar	0.24*	–0.94	–0.04*	0.08*	–0.51*	—
	(0.00–0.49)	(–1.28 – –0.60)	(–0.12–0.40)	(–0.05–0.21)	(–1.13–0.11)	—
Bluegill	0.40	1.36	–0.05*	0.03*	–0.15*	—
	(0.24 –0.55)	(1.12–1.60)	(–0.09–0.00)	(–0.03–0.08)	(–0.50–0.19)	—
White crappie	0.10*	–0.69	0.15	–0.04*	–0.30*	–0.48
	(–0.14–0.34)	(–0.99 – –0.40)	(0.09–0.21)	(–0.02–0.10)	(–0.66–0.06)	(–0.65 – –0.32)

Note: β_1, 30-year hydrographic β_2, temperature; β_3, microcrustacean density; β_4, isolation days; β_5, predator abundance; β_6, autocorrelation correction parameter.

*Parameters were found to have little support following calculation of unconditional confidence intervals.

Table 3. Results of generalized estimating equations comparing abundance of adults between habitats.

	All habitats		BR × OF		BR × OR		OF × OR	
Species	χ^2	P	χ^2	P	χ^2	P	χ^2	P
Mosquitofish	3.20	0.202	—	—	—	—	—	—
Red shiner	14.50	<0.001	14.19	<0.001	13.99	<0.001	2.24	0.135
Gizzard shad	18.14	<0.001	17.45	<0.001	4.29	0.038	13.80	<0.001
Spotted gar	12.64	0.002	7.85	0.005	12.91	<0.001	3.12	0.077
Longnose gar	12.89	0.002	13.05	0.002	13.32	<0.001	0.11	0.735
Bluegill	11.04	0.004	3.69	0.055	8.27	0.004	6.08	0.014
White crappie	17.69	<0.001	11.87	0.001	13.42	<0.001	2.92	0.087

Note: Significance was assessed at $\alpha = 0.013$ (Bonferroni-corrected). BR, Brazos River; OF, an oxbow lake that connects to the active channel relatively frequently at moderate levels of BR discharge (OXFREQ); OR, an oxbow lake that connects relatively rarely at high BR discharge (OXRARE).

with reproductive activity of species with divergent life history strategies. Five of the seven species examined had competing models that included well-supported associations with the 30-year hydrograph, whereas isolation days during the study period had little support in data for any species. This suggests that a subset of Brazos River fishes have reproductive ecologies adapted to long-term hydrologic dynamics, which supports the FPC model that stresses the importance of predictable flow periods to fish reproductive activity (Junk et al. 1989; Bayley 1991). The advantage of floodplain inundation should be greater when floods coincide with optimal temperatures (Gutreuter et al. 1999; Winemiller 2005; Schramm and Eggleton 2006). Over the 2-year study period, some floods occurred outside of species' reproductive periods (winter and fall), and this may have influenced the weak support for the short-term hydrologic predictor variable (isolation days).

Relationships between GSI and temperature suggested that species with different life history traits might exploit floods differently. Negative relationships between GSI and temperature were associated with species (longnose gar, gizzard shad, and white crappie) that had greater fecundity (mean fecundity > 13 000) and maximum body size (maximum SL > 250 mm). Reproductive activity of these species was greatest in spring as the 30-year hydrograph was increasing. This strategy would allow early life stages to move into slack-water floodplain habitats that are important nursery areas for some species (Sabo and Kelso 1991; Killgore and Baker 1996; Pease et al. 2006). Species with positive associations between GSI and temperature (mosquitofish, red shiner, and bluegill) had small adult sizes (maximum SL < 110 mm) and extended reproductive periods (5–7 months). Reproductive activity of these species coincided with peak flows that may allow them to colonize off-channel floodplain habitats and exploit them for reproduction during low-flow periods. King et al. (2003) reported that mosquitofish were one of the few species that revealed an increase in juvenile abundance in response to flooding in an Australian floodplain river. Only spotted gar had a negative association with the 30-year hydrograph. Adults of this species are common in backwater habitats and may reproduce during low-flow periods as described in the LFR model (Humphries et al. 1999).

Biotic predictor variables tended to have less support for most species and may have a greater effect on young-of-the-year survival, whereas abiotic variables had a greater influence on reproductive activity. Despite this pattern, several well-supported associations with biotic predictors were iden-

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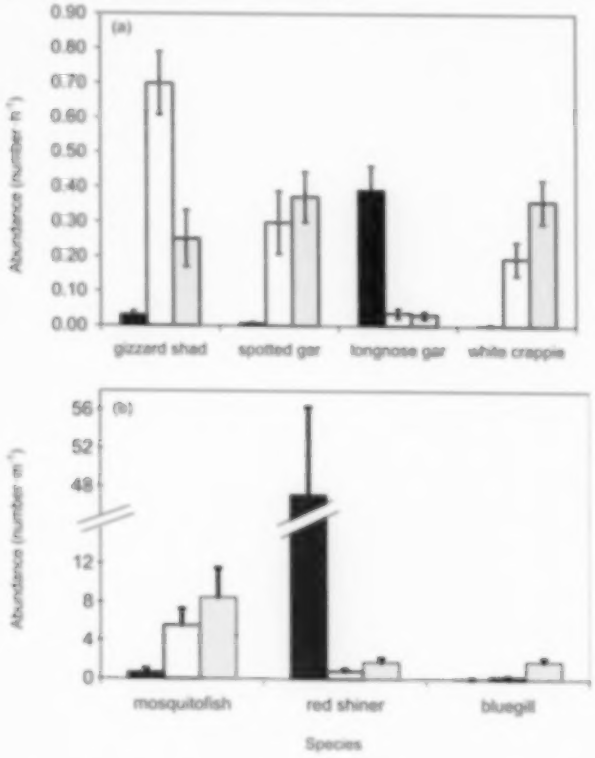
Fig. 5. Mean abundances and standard errors for adults of the seven selected species in each habitat. (*a*) Abundances of large species captured in gill nets. (*b*) Abundances of small species captured with seines. Solid bars, Brazos River; open bars, OXFREQ (an oxbow lake that connects to the active channel relatively frequently at moderate levels of Brazos River discharge); shaded bars, OXRARE (an oxbow lake that connects relatively rarely at high Brazos River discharge).



tified. Crappie and shad had positive associations with microcrustacean density that were strongly supported by data. The density of food resources for larvae is important to the production of strong year classes for species that produce many small offspring (Winemiller and Rose 1993), and shad and crappie had the greatest mean fecundity and smallest eggs of the seven species examined. Gizzard shad and red shiner had negative associations with predator abundance, whereas mosquitofish had a positive association. Zeug et al. (2005) found that red shiner abundance was significantly influenced by predator abundance in a Brazos oxbow, and juvenile gizzard shad are a common food item of Brazos predators (S.C. Zeug, unpublished data). Mosquitofish are able to exploit shallow habitats that may allow them to reproduce successfully despite greater predator abundance.

Copp (1989) suggested that patterns of adult abundance are good indicators of the reproductive function of a habitat. Evaluation of adult abundance patterns in the Brazos River suggested that oxbow lakes were particularly important for equilibrium strategists (crappie, bluegill) that were rarely collected in the main channel where frequent sub-bank-full flows may disrupt reproductive activity. Crappie and bluegill are nest-building brood guarders, and reproduction in the channel may be less successful during flood conditions. Bonvechio and Allen (2005) found negative associations between flow rate and year class strength of equilibrium species (centrachids) in Florida rivers. Periodic species were abundant in Brazos River channel and floodplain habitats. The larger size and greater longevity of periodic species allow them to survive during suboptimal periods, and although some reproduction probably occurs every year (Humphries et al. 2002), strong year classes may be produced only when floods coincide with optimal temperatures (Sommer et al. 2001; Halls and Welcomme 2004). Opportunistic strategists were abundant in all habitats, and these species may be able to reproduce successfully in a variety of habitats and under a wide range of environmental conditions (Winemiller 1989; Spranza and Stanley 2000). Some species may use multiple habitats during their life cycle (Schiemer 2000); however, among the seven species surveyed in the Brazos, there was no obvious segregation between adult and juvenile habitats.

Observed patterns of reproductive activity and habitat occupancy suggest that both flood dynamics and habitat heterogeneity are important for fish reproduction in rivers. Pease et al. (2006) found that both flood timing and the presence low-velocity, off-channel habitats were important for larval and juvenile fishes in the Rio Grande, New Mexico. Instream flow methodologies that focus on historical flow characteristics, such as the range of variability approach proposed by Richter et al. (1997), may be superior to other methodologies (Jowett 1997) because fish reproductive ecologies appear to be adapted to long-term hydrologic dynamics. Off-channel floodplain habitats, such as oxbow lakes, sloughs, and marshes, provide valuable spawning and nursery areas for many fish species (Sabo and Kelso 1991; Killgore and Baker 1996; Swales et al. 1999) and appear to be particularly important for equilibrium-type species (Kwak 1988; Scott and Nielsen 1989). Most instream flow strategies focus on in-channel or seasonally flooded habitats (Jowett 1997); however, perennial slack water areas also require consideration. Construction of levees or alteration of fluvial processes that drive the creation and succession of off-channel habitats would likely result in reduced productivity of species associated with these habitats (Aarts et al. 2004).

Species responses to flow regulation and flood management strategies may be predicted in the context of life history – environment relationships (Olden et al. 2006); however, caution should be used in the application of these results. Only 7 of 45 species collected (16%) were included in our study. The three endpoint model of Winemiller and Rose (1992) describes a gradient, and certain species have characteristics that would place them at intermediate positions between endpoint strategies. Some species have highly specific physicochemical or other habitat requirements for reproduction that may be obscured by analyses at the level of life history strategy. Only three habitats were surveyed for adult abundance estimates over the 2-year study; however, a previous survey of 10 Brazos oxbows by Winemiller et al. (2000) indicated that habitats with similar geomorphic characteristics supported similar abundances of species with similar life history traits.

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Large rivers display a wide range of hydrologic dynamics and spatial habitat units that influence aquatic fauna (Puckridge et al. 1998; Thorp et al. 2006). Alteration of either of these components can substantially reduce fish populations adapted to natural fluvial dynamics (Moyle and Light 1996; Aarts et al. 2004). Consideration of how alternative life history strategies respond to flow and habitat features of fluvial systems can increase the utility of conceptual models as guides for flow management and habitat restoration strategies.

Acknowledgements

We thank D. Lightsey and J.T. Lyons for allowing us access to their land during the study. Ray Matthews and Tim Osting (TWDB) provided oxbow connectivity estimates. We are grateful to C. Robertson, V. Shervette, J. Putegnat, J. Muir, D. Perretti, D. Hoeinghaus, and J. Birnbaum for their assistance in the field. Funding for this study was provided by TWDB. During the study, SCZ was supported by the Texas A&M Merit Fellowship, Texas Water Resources Institute Mills Scholarship, and the Tom Slick Senior Graduate Fellowship. Collections were made under Texas Parks and Wildlife Department permit No. SPR-0193-580.

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Spatial and temporal variation in shark communities of the lower Florida Keys and evidence for historical population declines

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Abstract: Sharks are top predators in many marine ecosystems. Despite recent concerns over declines in shark populations, studies of shark communities in coastal habitats are limited. We used drumlines and longlines to determine shark community composition and habitat affinities in the Florida Keys, USA. Community composition varied among habitats. Catch rates of smaller sharks were highest in protected shallow waters, while large sharks were more abundant in deep channels. Overall probabilities of catching large sharks on drumlines did not vary with water temperature, while catches of small sharks on longlines increased with increasing water temperature. Individual species differed in their responsiveness to variation in water temperatures and habitat. Bait type affected catch rates of some species, suggesting that fishing methods should be considered explicitly in studies describing shark communities or temporal trends in abundance. Catch rates of large-bodied sharks were higher in a remote and protected location compared with similar habitats near inhabited Keys. Also, historical accounts of a shark fishery in the study area during the 1920s suggest substantial declines in large shark abundance and shifts in community composition. By implication, ecosystem impacts of changes in the large shark community may be dramatic and likely occurred before adequate baselines were established.

Résumé : Les requins sont des prédateurs supérieurs dans plusieurs écosystèmes marins. Malgré les inquiétudes récentes sur le déclin des populations de requins, il y a peu d'études sur les peuplements de requins dans les habitats côtiers. Nous avons utilisé des lignes sur bouées (drumlines) et des palangres pour déterminer la composition des peuplements de requins et leurs préférences d'habitat dans les Keys de la Floride, É.-U. La composition du peuplement varie en fonction des habitats. Les taux de capture des petits requins sont les plus élevés dans les eaux protégées peu profondes, alors que les grands requins sont plus abondants dans les chenaux plus profonds. Les probabilités globales de capturer de grands requins avec des lignes sur bouées ne varient pas en fonction de la température de l'eau, alors que les captures de petits requins à la palangre augmentent avec la température de l'eau. Les diverses espèces diffèrent dans leurs réactions aux variations de la température de l'eau et de l'habitat. Le type d'appât utilisé affecte le taux de capture de certaines espèces, ce qui indique que les méthodes de pêche doivent être prises en compte de façon explicite dans les études qui cherchent à décrire les peuplements de requins ou à déterminer les tendances temporelles de leur abondance. Les taux de capture des requins de grande taille sont plus élevés dans les endroits éloignés et protégés que dans des habitats semblables près des Keys habités. De plus, des données historiques sur la pêche aux requins dans la zone d'étude dans les années 1920 laissent croire à un déclin important de l'abondance des grands requins et à des changements dans la composition du peuplement. Implicitement, les impacts sur l'écosystème des changements dans le peuplement de grands requins peuvent être considérables et ceux-ci se sont sans doute produits avant que des données de base suffisantes n'aient été obtenues.

[Traduit par la Rédaction]

Introduction

Large sharks are top predators in many marine ecosystems, with the potential to impact marine communities through direct and indirect interactions (see Heithaus 2004 for a review). For example, tiger sharks (*Galeocerdo cuvier*) in Western Australia influence the spatial distribution of numerous prey species at multiple spatial scales and may be critical in shaping the dynamics of the pristine seagrass community of Shark Bay (e.g., Heithaus 2005; Heithaus and

Received 25 August 2006. Accepted 21 May 2007. Published on the NRC Research Press Web site at cjfas.nrc.ca on 26 September 2007. J19498

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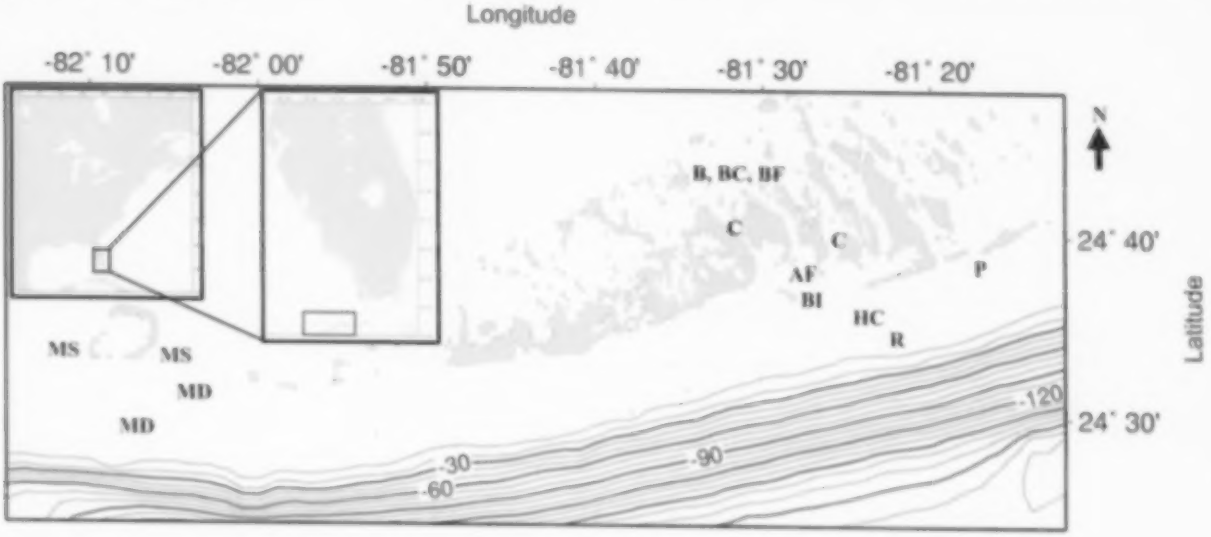
 doi:10.1139/F07-098 © 2007 NRC Canada

Fig. 1. The study site was located in the lower Florida Keys, including the Marquesas Islands. AF, Atlantic flat; B, backcountry (includes channels (BC) and flats (BF)); BI, barrier island; C, channel; P, passes; HC, Hawk Channel; MD, Marquesas deep; MS, Marquesas shallow; R, reef.



Dill 2006; Wirsing et al. 2007). However, ecosystem models suggest that in other situations, for example when there are other top predators (e.g., billfishes (Istiophoridae) and large tunas (*Thunnus* spp.)) present, declines in shark populations may not have a large influence on community structure (Kitchell et al. 2002).

Despite the potential importance of sharks in marine communities, there are relatively few quantitative studies published on their distribution, abundance, and community structure in coastal habitats. Such studies are of considerable importance at this time in light of likely declines in many populations (e.g., Baum et al. 2003; but see Baum et al. 2005; Burgess et al. 2005).

The waters surrounding the Florida Keys contain a diverse array of habitats for sharks of all life history stages. In this study, we investigated spatial variation in shark communities and the influences of habitat, water temperature, and bait type on catch rates of sharks in the coastal habitats of the lower Florida Keys. Because bait and gear types can influence catch rates (e.g., Heithaus 2001) or estimates of relative abundance, we used multiple bait types and two fishing techniques.

Materials and methods

Study site

The study was conducted in the lower Florida Keys offshore of Summerland and Big Pine Keys, approximately 40 km east of Key West, and in the Marquesas Keys, approximately 35 km west of Key West (Fig. 1). For the purposes of this study, we defined six habitats for drumline sampling (see below). The backcountry habitat is located north of Summerland Key and is characterized by very shallow waters, usually less than 1.5 m depth, that are cut by deeper channels (to 6 m). Numerous small, mangrove-lined keys are located throughout the habitat. Owing to gear limitations, drumline fishing was restricted to channels over 3 m depth. Several deep (to 9 m depth) passes between keys connect the shallow habitats north of the main keys with the waters to the south. These pass habitats often experience swift tidal currents. Hawk Channel (6–13 m depth) is situated inshore of the reef tract. Fishing in the reef habitat occurred along the reef edge, north of Looe Key Reef, in waters between 6 and 10 m depth. The relatively isolated and uninhabited Marquesas Keys are 35 km from the nearest human populations and have been protected as part of the Key West Wildlife Sanctuary since 1908. Although recreational fishing is allowed, their remote location leads to much lower levels of fishing and boating than the four habitats offshore of the densely populated Big Pine and Summerland Keys. The Marquesas shallow habitat was 3–4.5 m deep, and many sets were made near wrecks. The Marquesas deep habitat included waters south of the islands, inside the reef tract (similar to Hawk Channel) and in Boca Grande channel, east of the Marquesas, which experiences substantial water flow between the Gulf of Mexico and the Atlantic Ocean.

For studies using longlines (see below), we defined five habitats. Atlantic flats are expanses of shallow waters south of the main keys that are protected from the Atlantic Ocean by mangrove-lined barrier islands. Fishing in barrier island habitats occurred primarily in channels (2–4 m depth) close to the islands, while Atlantic flat habitats were more open. Passes and large channels leading to the Atlantic Ocean were combined into a single habitat (i.e., channel). The backcountry habitat described above was subdivided for longline studies into backcountry channels and backcountry flats.

Field methods

From November 2001 to July 2005, we sampled the large shark community using benthic drumlines. An eyebolt was embedded in a ~18 kg concrete weight. About 30 m of

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Table 1. Catches of sharks and rays on drumlines and longlines off the lower Florida Keys.

Common name	Scientific name	Drumline	Longline
Nurse shark	*Ginglymostoma cirratum*	173	103
Great hammerhead	*Sphyrna mokarran*	40	2
Blacktip shark	*Carcharhinus limbatus*	35	23
Bull shark	*Carcharhinus leucas*	27	0
Blacknose shark	*Carcharhinus acronotus*	14	18
Lemon shark	*Negaprion brevirostris*	6	70
Spinner shark	*Carcharhinus brevipinna*	2	0
Sandbar shark	*Carcharhinus plumbeus*	2	0
Tiger shark	*Galeocerdo cuvier*	2	0
Bonnethead	*Sphyrna tiburo*	1	35
Smalltooth sawfish	*Pristis pectinata*	0	3

3.5 mm monofilament line was attached to a swivel and shackled around the shank of the bolt. The line terminated in a second swivel that was attached to a 1.5 m double strand of 3.5 mm monofilament line attached to a Mustad 16/0 heavy duty tuna circle hook with offset point. A rope and buoys attached to the eye of the bolt marked the location of the line. This gear allows the shark to swim continuously once it is hooked, thereby minimizing stress to the animal.

Up to 20 lines were set within one or two habitats concurrently and allowed to soak for approximately 2 hours before they were retrieved. At each sampling site, we measured water temperature at midwater using a YSI 85 temperature, salinity, and dissolved oxygen meter. Each line was set up to five times in a single day. Because of limited availability of bait, we used multiple bait types, including elasmobranchs supplied by other projects that required destructive sampling, 1–2 kg portions of great barracuda (*Sphyraena barracuda*) and Atlantic bonito (*Sarda sarda*), frames from a variety of teleosts (e.g., snappers (Lutjanidae), groupers (Serranidae), mahimahi (Coryphaenidae)), and small, whole teleosts (primarily frozen bluefish, *Pomatomus salatrix*). Based on preliminary analyses using logistic regression, we combined barracuda and bonito into a single bait group (higher oil content) and other teleosts and elasmobranchs into a second group (lower oil content) for purposes of analysis.

Sharks were brought to the side of the boat and allowed to swim slowly forward to minimize capture stress. We measured precaudal length, fork length, total length (TL), and stretched total length (STL) of each individual; determined the sex; applied a rototag to the first dorsal fin; and released the shark.

From September 2003 to August 2005, we sampled the shark community with a ~300 m benthic longline made of 6.4 mm braided nylon rope. The longline was weighted with a danforth anchor on one end and a 2 kg lead weight on the other. Fifty gangions made of 6 mm nylon rope attached to wire leaders and a Mustad 14/0 circle hook with offset point were spaced evenly along the mainline. Hooks were baited with barracuda, striped mullet (*Mugil cephalus*), bonito, Atlantic thread herring (*Opisthonema oglinum*), and teleost frames. Preliminary analyses using logistic regression indicated no effect of bait type on catch rates of any species, so bait was not used as a factor in analyses of longline data. The line was soaked for 30–60 min.

Analyses

Transformations were unsuccessful in normalizing longline data and daily catch rate data from drumlines when bait type was included in analyses in large part because of the large number of zero values in the data set. Therefore, we used logistic regression to determine the influences of habitat, water temperature, and all two-way interactions on the probability that a longline set captured at least one shark or sawfish and the influences of habitat, water temperature, bait type, and relevant interactions on the probability of capturing a shark on a drumline. Final models were determined using a backwards stepping procedure. Nonsignificant ($P > 0.10$) interactions were removed first, followed by nonsignificant main effects ($P > 0.10$).

Community similarity was investigated using several indices that place differing weights on the presence of rare species and species relative abundances. These indices were selected to provide a diversity of perspectives on community similarity. Jacard's index compares the similarity of species composition using only species presence or absence data. Because this index can be heavily influenced by shared rare taxa, we only included species represented by more than 20 individuals. The proportional index of community similarity (PI) and Bray–Curtis index (BCI) integrate data on the relative abundances of each species and absolute species abundances, respectively. Therefore, the PI and BCI will cluster habitats with communities that share common taxa, while Jacard's index will tend to cluster habitats with similar species compositions.

Results

We made 1375 drumline sets on 77 days and 198 longline sets of 50 hooks on 79 days. Nurse sharks (*Ginglymostoma cirratum*) were the most commonly captured species on both gear types (Table 1). On drumlines, great hammerhead (*Sphyrna mokarran*), blacktip (*Carcharhinus limbatus*), and bull (*Carcharhinus leucas*) sharks also were common. In contrast, lemon sharks (*Negaprion brevirostris*) and bonnetheads (*Sphyrna tiburo*) were, respectively, the second and third most abundant species captured on longlines (Table 1).

Sharks caught on longlines generally were smaller than those captured on drumlines (Table 2). Significantly larger nurse ($t = 15.7$, df = 271, $P < 0.0001$), lemon ($t = 5.1$, df = 94, $P < 0.001$), and blacktip ($t = 8.5$, df = 57, $P < 0.0001$)

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Table 2. Mean lengths (stretched total length (STL) in cm) of sharks captured by drumline and longline in the Florida Keys.

	Male				Female			
	Drumline		Longline		Drumline		Longline	
Shark species	Mean±SD (range)	*n*	Mean±SD (range)	*n*	Mean±SD (range)	*n*	Mean±SD (range)	*n*
Nurse	235.5±22.8 (127–361)	94	146.6±36.9 (70–245)	52	230.0±22.5 (152–262)	69	142.4±27.2 (102–210)	42
Great hammerhead	257.9±25.9 (199–320)	22	(197–250)	2	275.8±43.6 (222–370)	18	—	—
Blacknose	111.3±8.4 (97–120)	7	100.3±11.2 (94–118)	3	110.9±7.4 (96–121)	9	108.9±12.4 (82–124)	13
Blacktip	152.4±7.7 (132–165)	18	80.1±16.7 (74–164)	11	159.7±18.2 (110–182)	17	95.2±32.9 (63–170)	10
Lemon	234.0±32.1 (197–255)	3	107.7±33.6 (81–215)	22	193.0±24.8 (165–209)	3	137.4±40.4 (66–223)	46
Bull	219.1±22.9 (178–249)	10	—	—	220.8±33.9 (176–275)	15	—	—
Bonnethead	—	—	—	—	—	—	82.3±8.0 (57–94)	25

Note: Sample sizes are the number of individuals reliably measured. SD, standard deviation.

sharks were captured on drumlines, but blacknose sharks (*Carcharhinus acronotus*) were of similar size on both gear types ($t = 0.34$, df = 33, $P = 0.63$). Based on calcification of their claspers, a large proportion of nurse and blacktip shark males captured on drumlines was mature. Nurse shark males mature at around 220 cm, and 71.1% of the males caught on drumlines were mature. Similarly, all male blacktip sharks caught on drumlines for which data were recorded (>141 cm) were mature. In contrast, only 26.7% of male blacktips and 21.7% of male nurse sharks caught on longlines were mature. Only 4 of 10 male bull sharks were mature, and all were over 230 cm. Male great hammerheads ranged from 199 to 320 cm STL. One 280 cm TL male had calcified claspers, as did all four animals over 300 cm STL. Four male sharks 281–295 cm STL had uncalcified claspers, suggesting that maturity occurs at ~300 cm.

There was significant spatial variation in the mean size of bull sharks ($F_{[4,24]} = 6.7$, $P = 0.001$). Sharks in the Marquesas shallows showed little size variation (mean ± standard error (SE) = 195.4 ± 2.4 cm STL, range = 189–205 cm, $n = 13$) and were significantly smaller than those found in the reef (247.2 ± 6.3 cm STL, range = 230–275 cm, $n = 7$) and pass habitats (230.3 ± 26.9 cm STL, range = 178–246 cm, $n = 4$). There was no spatial variation in the size of great hammerhead ($F_{[4,38]} = 2.3$, $P = 0.07$), nurse ($F_{[5,150]} = 1.2$, $P = 0.31$), and blacktip ($F_{[3,34]} = 0.1$, $P = 0.95$) sharks captured on drumlines.

Community composition

The species composition of sharks captured on both drumlines and longlines varied with habitat, but nurse sharks were the dominant species in all habitats except for backcountry channels sampled by longlines, where lemon sharks were most common (Figs. 2, 3). Jacard's index, which clusters communities based on species presence or absence, tended to cluster sampling areas in close proximity (Fig. 4*a*) rather than by sampling method or similarity of the habitat type, with the exception of Hawk Channel and the Marquesas deep, which are about 70 km apart but are similar habitat types. The proportional index, which uses data on species relative abundances, showed a high degree of community similarity when nurse sharks were included compared with analyses of only more mobile species (Figs. 4*b*, 4*c*). Both of these analyses tightly clustered Hawk Channel and Marquesas deep. The other deepwater habitats south of the main keys also clustered with these habitats. A second major cluster included the shallower habitats found to the north of the keys and those found south of the keys but inside barrier islands. The shark community of the shallow waters of the Marquesas was very distinctive because of the high abundance of juvenile bull sharks. Interestingly, the BCI (Fig. 4*d*), which uses species absolute abundance data, tended to cluster habitats of similar type (e.g., Atlantic flats and backcountry flats) more closely than habitats of close proximity that were closely linked by the proportional index (e.g., backcountry flats and backcountry channel).

Catch rates

The predicted probability of capturing a shark on a drumline was influenced by habitat and bait type, but not by water temperature (Table 3). The probability of catching a

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Fig. 2. Spatial variation in relative abundance of sharks caught on drumlines in the lower Florida Keys. Inset chart shows overall species composition. MS, Marquesas shallow; MD, Marquesas deep; B, backcountry; HC, Hawk Channel; R, reef; P, passes.

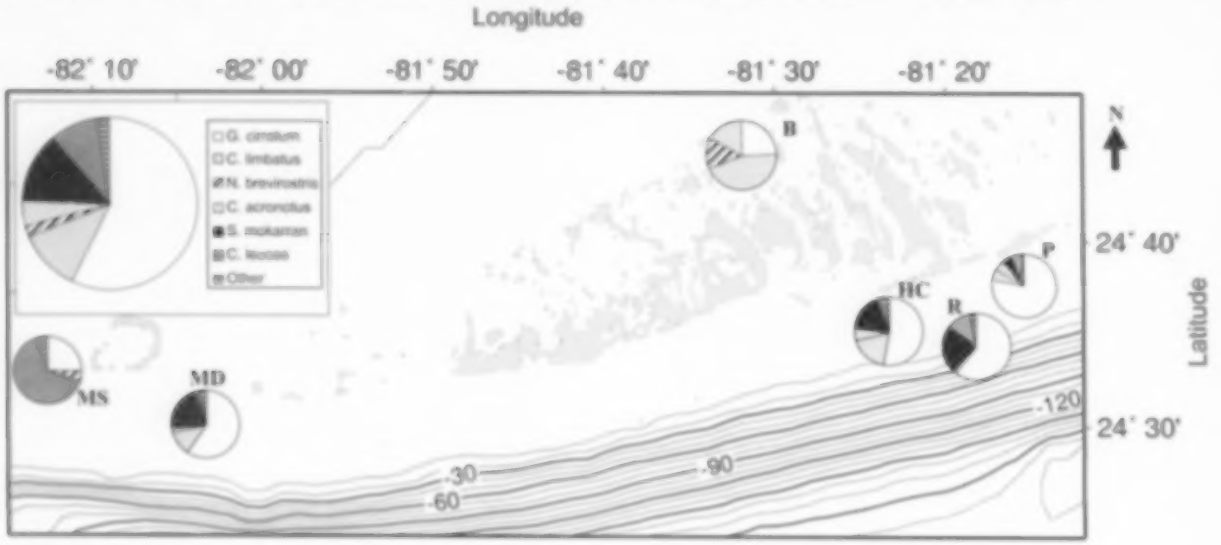
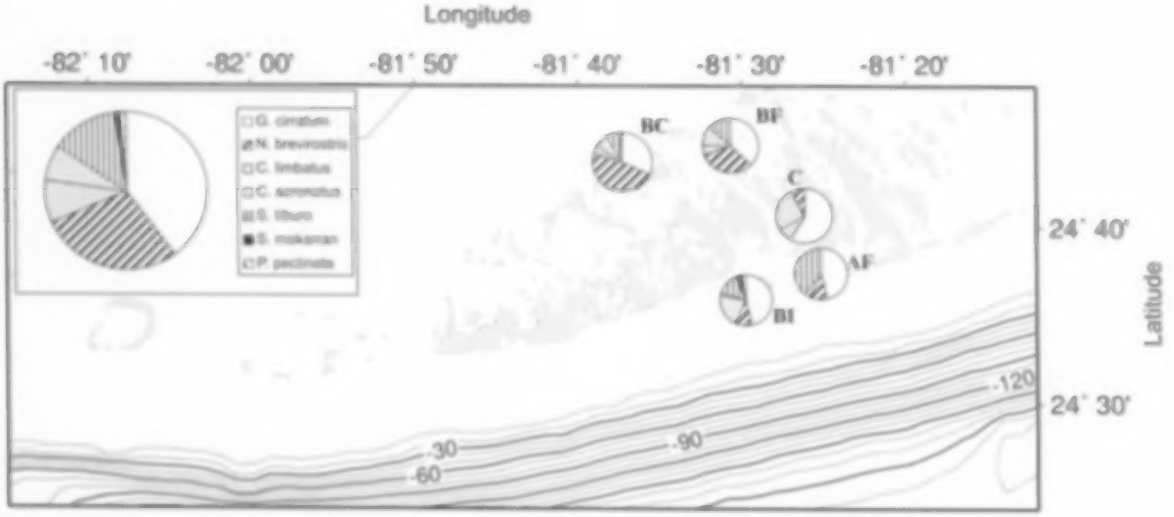


Fig. 3. Spatial variation in relative abundance of sharks and smalltooth sawfish (*Pristis pectinata*) caught on longlines in the lower Florida Keys. Inset chart shows overall species composition. BC, backcountry channels; BF, backcountry flats; C, channel; AF, Atlantic flat; BI, barrier island.



shark was highest in both Marquesas habitats and lower in the habitats off Summerland Key (Fig. 5). Barracuda and bonito were predicted to have higher probabilities of catching a shark (predicted probability = 0.26) than elasmobranch and other teleost baits (predicted probability = 0.19).

There was variation in the factors influencing predicted capture probabilities of individual species. The probability of catching a nurse shark on a drumline was influenced by an interaction of habitat and water temperature (Table 3). Nurse sharks were most likely to be caught in deep habitats of the Marquesas and passes and least likely to be captured in the backcountry (Fig. 6*a*). Predicted catch probabilities increased with rising water temperatures in all habitats, but the increase was greater in the deep habitats of the Marquesas than in the backcountry (Fig. 6*a*). Like nurse sharks, the predicted probability of capturing a great hammerhead was influenced by an interaction of habitat and water temperature but also by bait type (Table 3). Bonito and barracuda are predicted to capture twice as many great hammerheads (predicted probability = 0.04) as other baits (predicted probability = 0.02). The probability of catching a great hammerhead was highest in the deep habitats of the

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Fig. 4. Similarity of shark communities sampled by longline and drumline based on (*a*) Jacard's index, the proportional index of community similarity with nurse sharks (*Ginglymostoma cirratum*) included (*b*) and excluded (*c*) from analyses, and (*d*) the Bray–Curtis index.

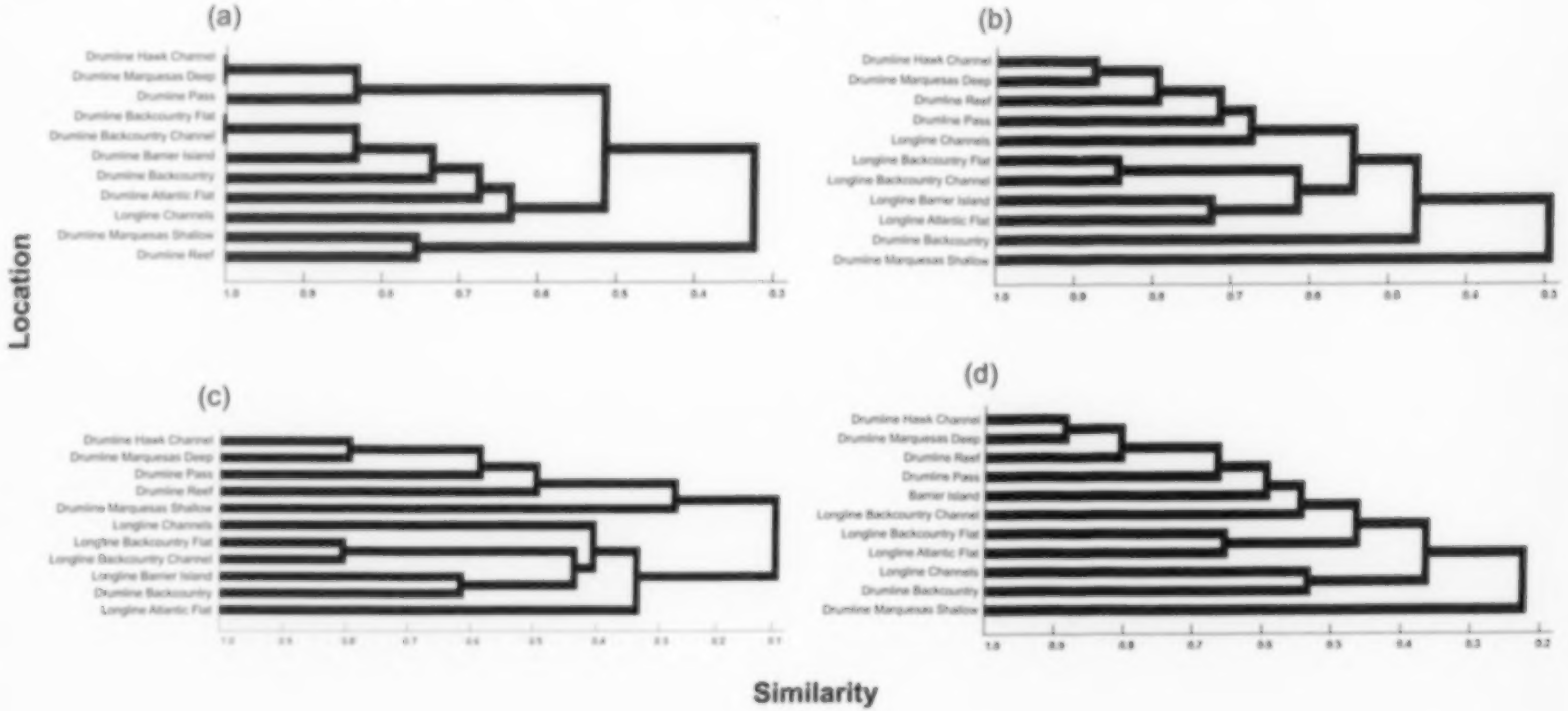

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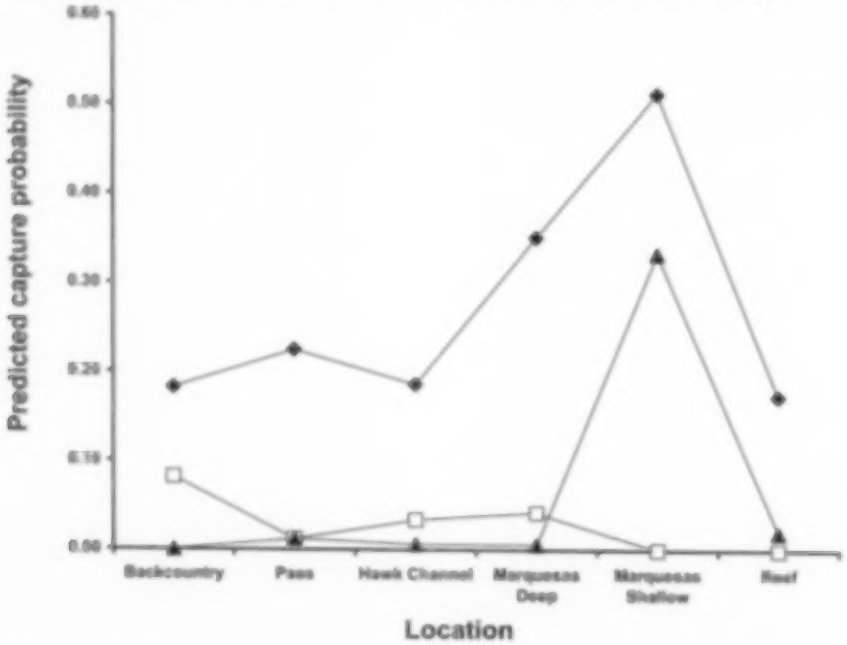
Table 3. Summary of logistic regression for predicting captures of sharks on drumlines (n = 1375 for final models).

Terms	Wald χ^2	P
All sharks		
Habitat	40.4	<0.0001
Bait	9.8	0.002
Whole model: log likelihood = 726.3; Wald χ^2 = 49.3, P < 0.0001; R^2 = 0.034, df = 6		
Nurse shark		
Habitat	18.1	0.003
Water temperature × habitat	15.0	0.01
Whole model: log likelihood = 518.3; Wald χ^2 = 47.3, P < 0.0001; R^2 = 0.046, df = 10		
Great hammerhead		
Bait	10.4	0.001
Water temperature × habitat	35.0	<0.0001
Whole model: log likelihood = 180.9; Wald χ^2 = 59.7, P < 0.0001; R^2 = 0.17, df = 11		
Bull shark		
Habitat	72.4	<0.0001
Whole model: log likelihood = 132.9; Wald χ^2 = 67.5, P < 0.0001; R^2 = 0.25, df = 5		
Blacktip shark		
Habitat	13.0	0.02
Whole model: log likelihood = 163.0; Wald χ^2 = 38.3, P < 0.0001; R^2 = 0.11, df = 5		

Note: Only factors with P < 0.10 are included.

Fig. 5. Spatial variation in the predicted probability of catching any shark (solid diamonds), a blacktip shark (*Carcharhinus limbatus*, open squares), or a bull shark (*Carcharhinus leucas*, solid triangles) on a drumline in the lower Florida Keys.



Marquesas followed by Hawk Channel and reef habitats (Fig. 6*b*), and this species was never captured in the backcountry or shallow habitats of the Marquesas. In general, the probability of catching a great hammerhead fell with increasing water temperature (Fig. 6*b*). Blacktip and bull shark catches on drumlines only varied with habitat (Table 3). The predicted probability of catching a blacktip shark was twice as high in the backcountry than in any other habitat (Fig. 5), and bull sharks were predicted to be captured much more frequently in shallow waters of the Marquesas than any other habitat. No bull sharks were caught in the backcountry.

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Fig. 6. The effects of water temperature and habitat on the predicted probability of catching (*a*) a nurse shark (*Ginglymostoma cirratum*) or (*b*) a great hammerhead (*Sphyrna mokarran*) on a drumline.

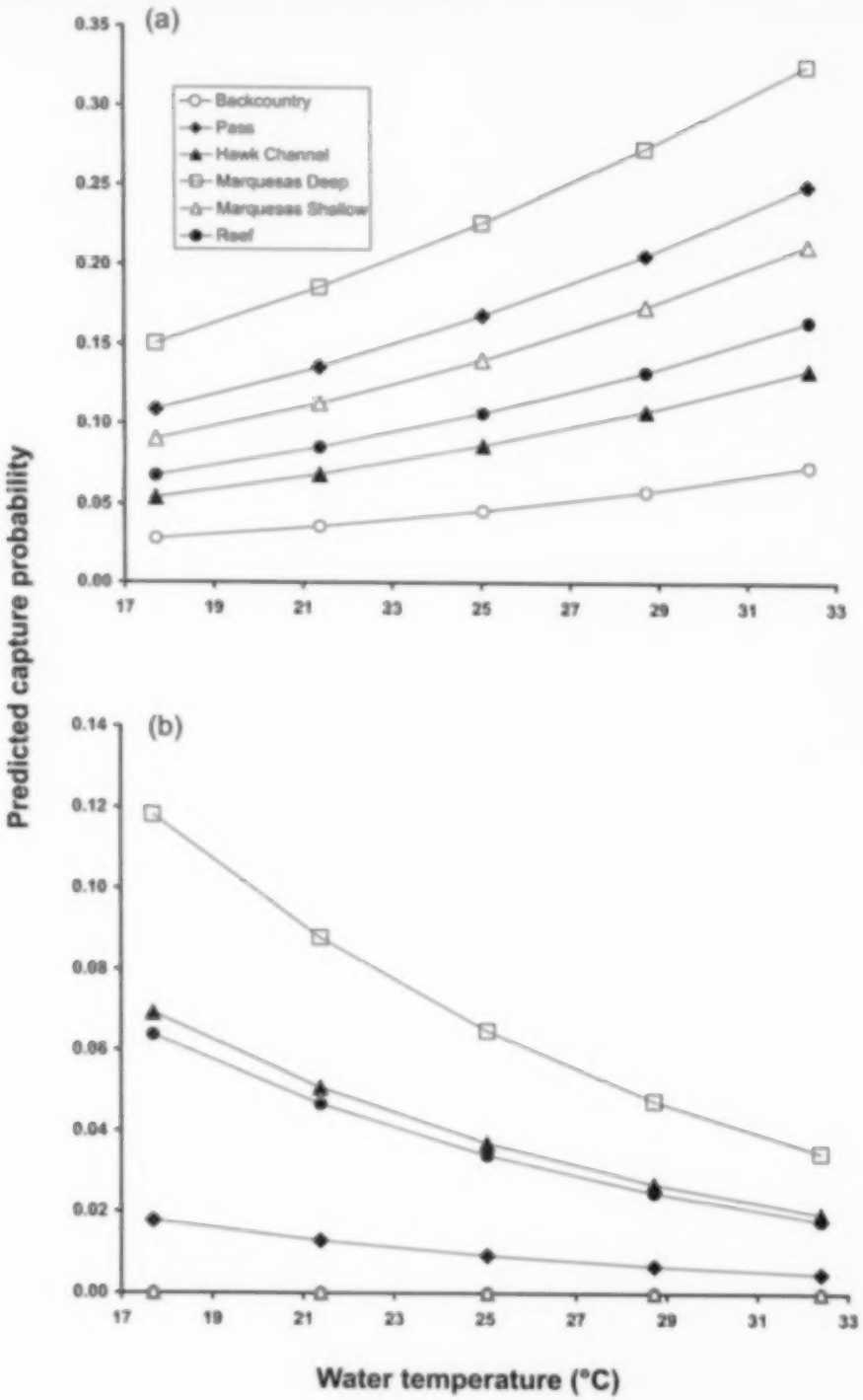

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Table 4. Summary of logistic regression for predicting captures of sharks on longlines (n = 198 for final models).

Terms	Wald χ^2	P
All sharks		
Water temperature	9.0	0.002
Whole model: log likelihood = 130.7; Wald χ^2 = 9.5, P = 0.002; R^2 = 0.04, df = 1		
Nurse shark		
Water temperature	9.1	0.003
Whole model: log likelihood = 123.8; Wald χ^2 = 7.4, P = 0.007; R^2 = 0.03, df = 1		
Lemon shark		
Habitat	13.8	0.008
Water temperature	9.5	0.002
Whole model: log likelihood = 90.8; Wald χ^2 = 24.3, P = 0.0002; R^2 = 0.13, df = 5		
Blacktip shark		
Habitat	9.5	0.05
Water temperature	3.7	0.05
Whole model: log likelihood = 53.1; Wald χ^2 = 13.0, P = 0.02; R^2 = 0.12, df = 5		
Blacknose shark		
Habitat	9.5	0.05
Whole model: log likelihood = 58.0; Wald χ^2 = 10.7, P = 0.03; R^2 = 0.09, df = 4		

Note: Only factors with $P < 0.10$ are included. No factors had $P < 0.10$ for bonnetheads.

The predicted probability of catching an elasmobranch during a longline set was influenced only by water temperature (Table 4), with probabilities increasing as water temperature rises (Fig. 7). Unlike catches on drumlines, the predicted probability of catching a nurse shark did not vary significantly with habitat, but increased with water temperature (Table 4, Fig. 7). The predicted probability of catching a lemon shark was highest in backcountry habitats, especially backcountry channels (Table 4, Fig. 8) and increased with rising water temperature (Fig. 7). The probability of catching a blacktip shark on a longline was greatest near barrier islands and lowest on Atlantic flats (Table 4, Fig. 8). The probability of catching blacktip sharks decreased with increasing water temperature (Fig. 7). Bonnethead capture probabilities did not vary spatially or with water temperature.

Discussion

The shark communities of the lower Florida Keys are dominated by nurse sharks. This pattern is not surprising given the nurse shark's ability to forage in areas of high structural complexity, which are common in the study area, because of its flexibility and suction-feeding abilities (e.g., Motta 2004). The dominance of nurse sharks also may be the result of historical population declines of other shark species (see below). Their low movement rates, relative to other shark species in the area, probably led to lower levels of exploitation in commercial gillnet fisheries that were once common in the study area (e.g., Viele 1996).

Despite being fished in similar locations, there were notable differences in the species composition of catches on drumlines and longlines. This disparity may arise from differences in gear types used, but appears to largely reflect *size* differences in habitat use by some common species. For example, adult lemon sharks appear to use the study area only rarely, while small lemon sharks are common, especially in backcountry habitats. Conversely, the youngest size classes of bull sharks are not present in the lower Florida Keys. Instead, they inhabit rivers and estuaries of the Florida Peninsula (e.g., Simpfendorfer et al. 2005). At intermediate sizes (150–200 cm), bull sharks appear to move into shallow coastal habitats like Florida Bay (Torres et al. 2006) and the shallow waters of the Marquesas Islands. Finally, at larger sizes (>200 cm), bull sharks begin using more open water habitats, like the reef and passes.

The probability of catching a shark on a drumline was not influenced by water temperature. However, there was a significant effect of the interaction between habitat and water temperature on the probability of capturing nurse sharks and great hammerheads. Catch probabilities for *G. cirratum* increased with rising water temperatures in all habitats, but increased fastest in deep habitats of the Marquesas. Conversely, *S. mokarran* catch probabilities in all habitats where they were caught were greatest during periods when water temperatures were coldest. No great hammerheads were caught in backcountry habitats and shallow waters of the Marquesas. The increase in catch rates of *S. mokarran* during the winter may be due to the seasonal southward movement of individuals from the colder waters of the northern Gulf of Mexico and northwest Atlantic Ocean during this time period. Indeed, many species are known to migrate south along the east coast of the United States and west coast of Florida as water temperatures drop (e.g., Kohler et al. 1998; Hueter et al. 2005). The absence of significant correlations between water temperature and catch rates for other species, especially large blacktip sharks, was somewhat surprising and could be due to generally low catch rates of most species other than nurse sharks, which are not known to be highly migratory.

Water temperature was an important determinant of catch probabilities on longlines. Capture probabilities overall and for nurse and lemon sharks in particular increased greatly as

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Fig. 7. Influence of water temperature on the predicted probabilities of catching any elasmobranch (solid triangles), a lemon shark (*Negaprion brevirostris*, solid diamonds), a blacktip shark (*Carcharhinus limbatus*, open squares), or a nurse shark (*Ginglymostoma cirratum*, open diamonds) on a longline set.

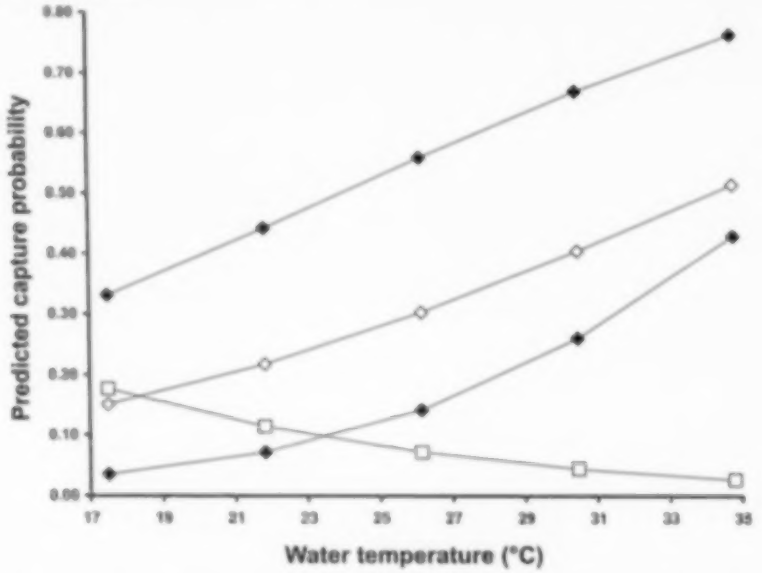
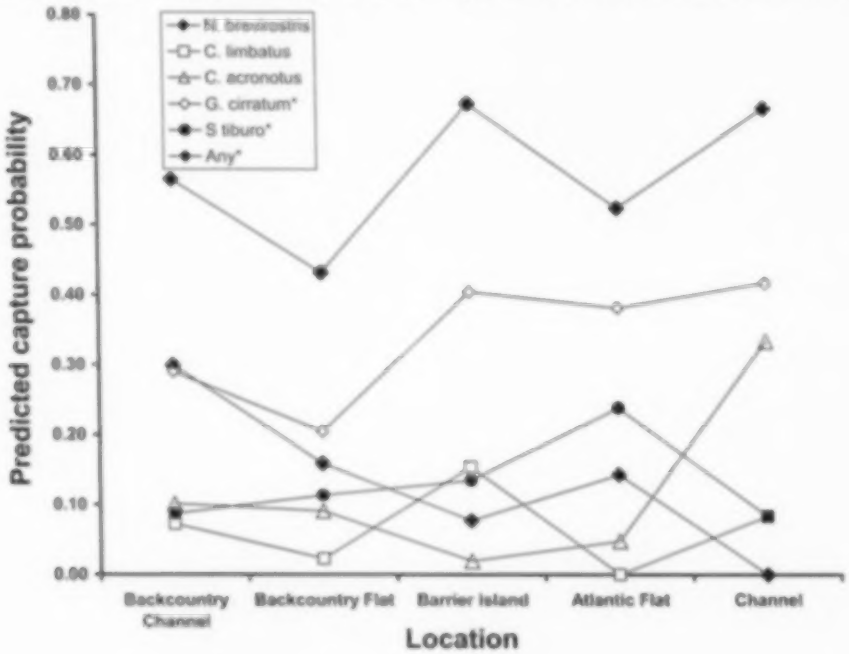


Fig. 8. Spatial variation in the predicted probability of catching any elasmobranch (closed triangles), a lemon shark (*Negaprion brevirostris*, solid diamonds), a blacktip shark (*Carcharhinus limbatus*, open squares), a blacknose shark (*Carcharhinus acronotus*, open triangles), a nurse shark (*Ginglymostoma cirratum*, open diamonds), or a bonnethead (*Sphyrna tiburo*, solid circles) on a longline set. Predicted capture probabilities do not vary significantly ($P < 0.05$) among habitats for taxa marked with an asterisk (*).



water temperature increased. For blacktip sharks, however, predicted catch probabilities decreased with increasing water temperatures. This pattern is consistent with a northward movement of blacktip sharks into the Gulf of Mexico to spend summer months in nursery habitats where they were born (Hueter et al. 2005).

The habitat use patterns of sharks in this study raise the possibility that large sharks, like bull sharks and great ham-

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merheads, influence habitat use of smaller species. Both bull sharks and great hammerheads consume small sharks (e.g., Cliff and Dudley 1991; Cliff 1995). Neither of these species was captured in backcountry habitats, where densities of small sharks, especially blacktip and lemon sharks, were highest. Therefore, habitat use by these juvenile sharks may be driven, at least partially, by low predator density. In general, the need to minimize predation risk, rather than to gain access to food, is gaining recognition as perhaps the primary determinant of habitat use by many species of juvenile sharks (see Heupel and Hueter 2002; Heithaus 2004, 2007).

Our finding that bait type influenced catch rates of some species on drumlines but not others has important implications for studies of shark communities. If studies make use of a single bait, then community composition of the catch may not reflect true relative abundances. Therefore, studies that make use of multiple bait types may be more effective in determining relative abundances. Describing shark community structure, however, remains difficult because of other gear-related biases, such as changes in species composition and size structure with gear type. For example, the composition of the community captured on drumlines in backcountry channels was different than that captured on longlines in the same location despite only small differences in hook sizes used and similar bait types. Therefore, variation in gear type and fishing method will likely modify catch rates in many circumstances, and empirical studies of the effects of fishing techniques on catch rates of sharks would aid in interpreting fisheries data used to detect changes in population sizes (e.g., Baum et al. 2005; Burgess et al. 2005).

One of the most striking findings of this study was the relatively low catch rate of large sharks. On average, a 10 h day of drumline fishing effort with 10–20 lines resulted in the capture of two sharks, and on average fewer than one shark other than nurse sharks was captured per day. Accounts of Hydenoil Inc., a shark fishing company that operated offshore of Big Pine Key from 1923 to 1932, indicate that large sharks were more abundant at that time, and the study area's shark community was different historically (Viele 1996). In the early 1930s, for example, gillnet fishermen operating in and near our study area reportedly landed around 100 sharks every day. These sharks are reported to have averaged nearly 2.3 m in length with numerous larger animals. Although capture techniques are different, based on our data such catch rates of large sharks are likely unattainable today. Furthermore, tiger sharks and smalltooth sawfish (*Pristis pectinata*) were reported as common catches. Although we used different fishing methods, tiger sharks and other large-bodied species are readily caught on our gear (Heithaus 2001), and our study suggests that numbers of these two previously abundant species have been reduced markedly and, consequently, that they are now rare. Furthermore, it is likely that other large-bodied species, with the possible exception of nurse sharks, also have been greatly reduced. Thus, the abundance and community composition of large sharks appears to be dramatically different from those present historically in the Florida Keys. Although the implications of the apparent reductions in the abundance of large sharks and changes in species relative abundances are unknown, it is likely that declines in species like tiger sharks have already dramatically affected the marine communities of the Florida Keys (see Heithaus 2004). These effects are likely to continue for the foreseeable future and may hinder efforts to restore the ecosystem to a more natural state. Studies of the ecological role of sharks in relatively pristine ecosystems and historical reconstructions of pre-exploitation shark population sizes in the Caribbean Sea and Gulf of Mexico will be of great value to predicting the likely consequences of large shark reductions in the area.

Acknowledgements

We thank the Mote Marine Laboratory Center for Tropical Research staff at Summerland Key for their support and help with this project. We especially thank Pete Braisted, Barbara Smith, Wayne Giordano, and Erich Bartels and the many Center for Shark Research student interns who provided assistance. We thank Aaron Wirsing, Alejandro Frid, and two anonymous reviewers for comments on a previous draft of this manuscript and for statistical advice. Funding was provided by the National Geographic Expeditions Council. This publication also was supported partially by the National Sea Grant College Program of the United States Department of Commerce's National Oceanic and Atmospheric Administration under NOAA Grant No. R/MI-12 and NOAA grants to the Mote Marine Laboratory Center for Shark Research and the National Shark Research Consortium. The views expressed herein do not necessarily reflect the views of any of these organizations. Research was conducted under Florida Fish and Wildlife Commission Permits 01S-041A, 02R-041, 04SR-041, and 04SR-041A. This research was conducted under Institutional Animal Care and Use Committee authorization by Florida International University and Mote Marine Laboratory.

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Consumption of two exotic zooplankton by alewife (*Alosa pseudoharengus*) and rainbow smelt (*Osmerus mordax*) in three Laurentian Great Lakes

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Abstract: Introductions of the zooplankton *Bythotrephes longimanus* and *Cercopagis pengoi* into the Great Lakes have drawn attention surrounding their suitability as prey for zooplanktivorous fishes. We used gut contents and stable carbon isotopes to quantify differential consumption and selection of the exotics by alewife (*Alosa pseudoharengus*) and rainbow smelt (*Osmerus mordax*) in Lakes Erie, Michigan, and Ontario. The exotics were more often consumed by alewife (up to 70% of gut content biomass) than by smelt (up to 25% of gut content biomass). Measured stable carbon isotope ratios of fish and ratios predicted from mixing models confirmed that the "snapshot" descriptions of diet through gut contents were representative of longer-term diets. While *B. longimanus* generally was selected for (14 of 17 sampling dates), *C. pengoi* was not a preferred prey item. *Cercopagis pengoi* was sometimes a large component of alewife diet because of its high densities in the environment. The exotic zooplankton are more important for alewife than for smelt, and consumption of the cladocerans varies throughout the growing season and among lakes, generally related to patterns of exotic abundance. Effects associated with consumption of the exotics should be high in alewife-dominated systems invaded by *B. longimanus* or large numbers of *C. pengoi*.

Résumé : L'introduction des organismes zooplanctoniques *Bythotrephes longimanus* et *Cercopagis pengoi* dans les Grands Lacs a généré de l'intérêt pour la question de leur convenance comme proies pour les poissons zooplanctonophages. Nous utilisons des analyses du contenu du tube digestif et des isotopes stables de carbone afin de mesurer la consommation différentielle et la sélection de ces espèces exotiques par le gaspareau (*Alosa pseudoharengus*) et l'éperlan arc-en-ciel (*Osmerus mordax*) dans les lacs Erie, Michigan et Ontario. Ces espèces exotiques sont consommées plus fréquemment par le gaspareau (jusqu'à 70 % de la biomasse du contenu du tube digestif) que par l'éperlan (jusqu'à 25 % de la biomasse du contenu du tube digestif). Les rapports des isotopes stables de carbone mesurés sur les poissons et les rapports prédits par les modèles de mélange confirment que les descriptions « en instantané » du régime alimentaire fournies par les analyses du contenu du tube digestif sont représentatives des régimes alimentaires à plus long terme. Alors que *B. longimanus* est généralement une proie recherchée (14 des 17 dates d'échantillonnage), *C. pengoi* ne l'est pas. *Cercopagis pengoi* forme occasionnellement une partie importante du régime des gaspareaux à cause de ses fortes densités dans le milieu. Le zooplancton exotique est donc plus important pour les gaspareaux que pour les éperlans et la consommation de ces cladocères varie au cours de la saison de croissance ainsi que d'un lac à l'autre, généralement en fonction des patrons d'abondance de ces espèces exotiques. Les effets reliés à la consommation de ces espèces exotiques devraient être importants dans les systèmes dominés par les gaspareaux et envahis par *B. longimanus* ou contenant de fortes densités de *C. pengoi*.

[Traduit par la Rédaction]

Introduction

Biological invasion has for centuries been a threat to North American Great Lakes. Since the 1800s, at least 176 nonindigenous organisms have become established in the Laurentian Great Lakes, arriving commonly in the ballast water of transoceanic ships (Carlton and Geller 1993; Mills et al. 1993; Holeck et al. 2004). Many recent invaders ar-

Received 16 July 2006. Accepted 24 May 2007. Published on the NRC Research Press Web site at cjfas.nrc.ca on 26 September 2007. J19422

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 doi:10.1139/F07-096 © 2007 NRC Canada

rived from Eurasia, especially the fresh and brackish waters of the Ponto-Caspian Basin (Black, Caspian, and Azov seas; Mills et al. 1993; Ricciardi and MacIsaac 2000). Among these organisms have been the confamiliar (family Cercopagidae) predatory cladoceran zooplankton *Bythotrephes longimanus* (formerly *Bythotrephes cederstroemi*) and *Cercopagis pengoi*.

Bythotrephes longimanus initially was documented in Lake Huron in 1984 (Bur et al. 1986). By 1987, the cladoceran had established populations in the remaining Great Lakes (Lake Erie, 1985, Bur et al. 1986; Lake Ontario, 1985, Lange and Cap 1986; Lake Michigan, 1986, Lehman 1987; Lake Superior, 1987, Cullis and Johnson 1988). Since its introduction, *B. longimanus* has been associated with many changes in Great Lakes pelagic food webs. For example, reductions in *Daphnia* spp. abundance were linked with *B. longimanus* predation in Lake Michigan (Lehman and Cáceres 1993; Schulz and Yurista 1999). Berg and Garton (1988) correlated decreases in numbers of the native predator *Leptodora kindti* with peak *B. longimanus* abundance. In Lake Ontario, alewife (*Alosa pseudoharengus*) feeding shifted from a diet dominated by native copepods and cladocerans to one also containing large proportions of *B. longimanus* (Mills et al. 1992).

Over a decade after the introduction of *B. longimanus*, *C. pengoi* was documented in Lake Ontario (1998, MacIsaac et al. 1999). Following its initial introduction, *C. pengoi* spread rapidly to other Great Lakes. As a result, researchers also have become interested in the potential impacts of this cladoceran on pelagic food webs. For example, Benoit et al. (2002) found evidence that *C. pengoi* may be reducing native copepod production by direct predation on nauplii or by displacing adults into colder thermal strata.

Because of their small sizes, exotic zooplankton have received less attention than macrofaunal invaders. However, these organisms may be important in aquatic food webs as abundant prey for planktivorous fishes (e.g., Hewett and Stewart 1989). Further, because the predatory *B. longimanus* and *C. pengoi* have long, chitinous tail spines and are generally unavailable to gape-limited planktivores, the exotic zooplankton may compete with young fishes for food resources (Barnhisel and Harvey 1995; Schulz and Yurista 1999, Pothoven and Vanderploeg 2004). Much of the attention surrounding the invasions of *B. longimanus* and *C. pengoi* has been in response to their potential competition with young, zooplanktivorous fish species.

In addition to tail spines that increase their body lengths, the exotic cladocerans have a prominent dark eye (Burkhardt 1994; Grigorovich et al. 2000), making them easily noticeable prey for visual predators such as planktivorous fishes. Some studies have shown that *B. longimanus* and *C. pengoi* can be important components in the diets of adult Great Lakes planktivores, which are larger and less likely to be gape-limited than juvenile fish (e.g., Mills et al. 1992; Pothoven and Vanderploeg 2004; Parker-Stetter et al. 2005). However, this previous work investigated fish feeding ecology in single systems. The role these exotics play in pelagic food webs will depend on their relative abundances, which differ between lakes. For example, *B. longimanus* abundance is lower in Lake Ontario than in Lakes Michigan and Erie, whereas *C. pengoi* is less common in Lake Erie than in Lakes Michigan and Ontario (K.L. Schulz and C.E. Cáceres, unpublished data). Therefore, to evaluate broader impacts of the *B. longimanus* and *C. pengoi* introductions on planktivore feeding ecology in the Great Lakes and in water bodies that the cladocerans may invade in the future, comparisons of systems with different abundances of the exotics is essential. Further, few studies have quantified selection for these conspicuous invertebrates by adult Great Lakes planktivores (but see Pothoven and Vanderploeg 2004), and researchers have yet to compare potential preference over broad spatial scales or have yet to use several methods simultaneously to estimate diet. Although *B. longimanus* may negatively impact young fish through competition, the implications of both exotic invertebrates as novel prey for adult zooplanktivores is less clear. The degree to which planktivorous fishes consume these invasive zooplankton, and whether they are actively selected over native prey, may impact planktivore condition and ultimately production.

The objectives of this study were to (*i*) quantify consumption of *C. pengoi* and *B. longimanus* by alewife and rainbow smelt (*Osmerus mordax*) and (*ii*) evaluate potential selection of the exotic zooplankton by these opportunistic planktivores in Lakes Erie, Michigan, and Ontario. In food web studies, stable carbon isotope ratios often are used to evaluate the source of carbon to a consumer or prey item. Because stable carbon isotope ratios change little with increasing trophic level (approximately 1‰ per trophic transfer; Michener and Schell 1994), an animal retains the approximate isotopic signature of the primary carbon source. Therefore, these stable isotope signatures can be used to reconstruct the long-term diet of consumers. We employed stable carbon isotopes to corroborate the "snapshot" description of diet provided by gut content analyses and to characterize time-integrated diet composition. Using analyses of fish gut contents, stable carbon isotope analyses of fish and potential diet items, and prey selection indices, we evaluated the extent to which *B. longimanus* and *C. pengoi* are consumed by alewife and rainbow smelt, and whether the exotics are preferred prey, in nearshore regions of Lakes Erie, Ontario, and Michigan during the 2002 and 2003 growing seasons (May–October).

Materials and methods

Field sampling

Adult fish and benthic and pelagic invertebrates were sampled during May–October 2002 and 2003 from nearshore and offshore waters of eastern Lake Erie near Dunkirk, New York; southwestern Lake Michigan near Zion, Illinois; and southern Lake Ontario near Brockport, New York (Table 1).

Invertebrates were collected monthly from long-term sampling stations established by the New York State Department of Environmental Conservation (Region 7; Lake Erie), the Illinois Natural History Survey (Lake Michigan), and State University of New York at Brockport (J. Makarewicz; Lake Ontario). For consistency, and to facilitate specific analyses (see below), fish were collected from waters close to these sites, at similar depths, and whenever possible, concurrently (i.e., on the same day) with invertebrate sampling.

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Table 1. Fish sampling dates, times, gear, and site descriptions for cruises during May–October 2002 and 2003 in Lakes Erie, Michigan, and Ontario.

Great Lake	Sampling date	Sampling time of day	Gear type	Fishing depth (m)	Latitude	Longitude
2002						
Erie	10–11 July	PM–AM	MT	8.7–13.5	42°24′29″–42°28′2″	79°5′43″–79°12′32″
	22 Oct.	AM	BT	27.5–30.0	42°19′41″–42°22′11″	79°12′52″–79°15′37″
Michigan	21 May	AM	BT	10.0	42°25′37″–42°13′16″	87°28′31″–87°28′34″
	18 June	AM	BT	10.0	42°25′37″–42°13′16″	87°28′31″–87°28′34″
	26 July	AM	BT	23.0	42°13′46″–42°14′5″	87°26′43″–87°26′42″
	20 Aug.	AM	BT	3.0	42°13′23″–42°13′40″	87°29′1″–87°29′6″
	12 Sept.	AM	BT	10.0	42°25′37″–42°13′16″	87°28′31″–87°28′34″
Ontario	19–20 June	PM–AM	GN	0.0–5.0	43°13′2″	77°32′58″
	24–25 July	PM–AM	GN	0.0–5.0	43°13′2″	77°32′58″
	13–14 Aug.	PM–AM	GN	0.0–5.0	43°13′2″	77°32′58″
	16–17 Sept.	PM–AM	GN	0.0–5.0	43°13′2″	77°32′58″
2003						
Erie	14–15 July	PM–AM	MT	10.5–18.0	42°21′32″–42°24′4″	79°7′6″–79°12′6″
	23 Oct.	AM	BT	19.6–20.0	42°26′22″–42°26′6″	79°3′55″–79°3′51″
Michigan	20 May	AM	BT	10.0	42°25′37″–42°13′16″	87°28′31″–87°28′34″
	16 June	AM	BT	10.0	42°25′37″–42°13′16″	87°28′31″–87°28′34″
	25 July	AM	BT	23.0	42°13′46″–42°14′5″	87°26′43″–87°26′42″
	15 Aug.	AM	BT	3.0	42°13′23″–42°13′40″	87°29′1″–87°29′6″
	25 Sept.	AM	BT	7.5	42°13′22″–42°13′40″	87°28′47″–87°28′47″
Ontario	14–15 May	PM–AM	GN	0.0–5.0	43°13′2″	77°32′58″
	11 June	AM	BT	15.0–75.0	43°19′9″–43°19′39″	76°13′40″–76°17′1″
	19–20 June	PM–AM	GN	0.0–5.0	43°21′71″	77°54′99″
	17–18 July	PM–AM	GN	0.0–5.0	43°21′73″	77°54′94″
	31 July	AM	BT	85.0–110.0	43°33′18″–43°34′05″	76°27′39″–76°30′98″
	18–19 Aug.	PM–AM	GN	0.0–5.0	43°21′73″	77°54′94″
	9–10 Sept.	PM–AM	GN	0.0–5.0	43°21′73″	77°54′94″

Note: MT, midwater trawl; BT, bottom trawl; GN, gill net. In Lake Ontario, the gill net was fished from the surface (headrope) to 5 m (leadline). Bottom and midwater trawls were fished at several depths throughout the ranges indicated.

Fish collection

Adult alewife and rainbow smelt were collected from eastern Lake Erie during July and October 2002 and 2003, southwestern Lake Michigan monthly during May–October 2002 and 2003, and southern Lake Ontario during June–September 2002 and 2003. The use of different gear types was required because of vessel capabilities (Table 1); however, this constraint ensured that the species of interest were captured during each sampling period. Samples sizes of fish analyzed on each date are provided (Table 2). In Lake Ontario, where sampling relied heavily in many months on nearshore gill nets, samples were collected during 2 months of 2003 with both nearshore gill nets and bottom trawls at a range of depths. Comparison of data from fish collected in the same month with these two gear types did not indicate important differences in diet composition. While different sampling gear is obviously not ideal, it was an unavoidable logistical constraint, and the similarity of diet of gillnet- and bottom-trawl-collected fish at least partially alleviates concern about sample gear bias in this study.

Alewife and rainbow smelt were collected from each lake in overnight gill net sets (Lake Ontario), daytime bottom trawls (Lakes Ontario, Erie, and Michigan), or nighttime midwater trawls (Lake Erie; Table 1). Upon capture, all fish were measured (total length (mm); Table 2). Samples were then quick-frozen on dry ice to minimize postcapture digestion and to preserve muscle tissue for later chemical analyses. In the laboratory, white muscle tissue was removed (1–5 mg dry weight) from an area above the lateral line, immediately behind the operculum. Muscle was excised from a random subsample for each species (approximately five fish per species per sampling date). Tissue samples were held in a –80 °C ultrafreeze (Ultra Low, Kendro Laboratory Products) prior to analyses.

Invertebrate collection

Predatory and herbivorous zooplankton were sampled in whole-water-column vertical tows of 500 µm (30 cm diameter) and 50 µm (20 cm diameter) Wisconsin high-efficiency plankton nets, respectively. During the May 2002 sampling in Lake Ontario and a June cruise on Lake Champlain during an algal bloom, a flow meter was used to estimate field efficiency of both the 500 and 50 µm nets. In addition, efficiencies of identical nets were tested in a flow tank, and results indicated that at towing speeds between 0.5 and 2 $m \cdot s^{-1}$ (bracketing our tow speed of 1 $m \cdot s^{-1}$), net efficiency was always higher than 95%. Because tank- and field-measured efficiencies always exceeded 95%, net efficiency for all subsequent sampling was assumed to be near maximal (i.e., 100%). Duplicate samples, used to quantify zooplankton densities, were collected with each net. Animals were then narcotized with carbon dioxide (antacid tablets containing

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Table 2. Total lengths (TL; mm ± standard deviation (SD)) and number of alewife (*Alosa pseudoharengus*) and rainbow smelt (*Osmerus mordax*) caught in Lakes Erie, Michigan, and Ontario during 2002 and 2003.

Great Lake	Month	Species	*n*	Total length (mm)
2002				
Erie	July	Alewife	20	145±10
	Oct.	Alewife	28	149±41
	July	Smelt	50	98±15
	Oct.	Smelt	20	118±22
Michigan	May	Alewife	4	78±7
	June	Alewife	17	110±23
	July	Alewife	18	126±19
	Aug.	Alewife	30	131±15
	Sept.	Alewife	15	112±12
Ontario	June	Alewife	40	152±9
	July	Alewife	23	147±16
	Aug.	Alewife	9	141±19
	Sept.	Alewife	13	149±11
2003				
Erie	Oct.	Alewife	24	103±14
	July	Smelt	20	101±17
	Oct.	Smelt	15	134±16
Michigan	May	Alewife	24	120±37
	June	Alewife	44	98±35
	July	Alewife	15	151±5
	Aug.	Alewife	11	125±32
	Sept.	Alewife	9	141±26
	June	Smelt	3	68±21
	July	Smelt	4	82±9
	Aug.	Smelt	1	79
Ontario	May	Alewife	30	156±15
	June	Alewife	52	130±29
	July	Alewife	36	142±14
	Aug.	Alewife	30	137±20
	Sept.	Alewife	4	148±30
	June	Smelt	45	104±10
	July	Smelt	36	127±34

Note: Sample sizes (*n*) are for fish analyzed for gut contents. Lengths were recorded for subsamples of alewife in Lake Michigan during June, August, and September of 2002 and June of 2003.

sodium bicarbonate) and preserved in 10% sucrose–formalin solution (Haney and Hall 1973). To obtain material for chemical analyses, further tows (independent of samples collected to estimate densities) were taken with each net. These zooplankton were poured through individual 50 µm Nitex mesh squares and frozen on dry ice. *Mysis relicta* were collected from Lake Ontario in daytime epibenthic sled tows. Upon capture, the animals were frozen on dry ice for chemical analyses.

Laboratory analyses

Fish gut content enumeration

Fish stomach contents were enumerated under a stereomicroscope (Leica MZ12.5, Leica Microsystems) following the procedure outlined by Mills et al. (1992). Digestive tracts, excluding the intestine, were removed from frozen fish and preserved in 10% buffered formalin. Fish guts were later rinsed of preservative and sectioned into anterior (esophagus) and posterior (stomach and pylorus) regions. The two limbs were then dissected, and the contents were rinsed into separate Petri dishes. Prey items were poured through a 50 µm mesh sieve and preserved in individual scintillation vials with buffered formalin.

Using standard taxonomic keys (Balcer et al. 1984; Pennak 1989; Thorp and Covich 1991), prey items were identified to species, with the exception of copepods and amphipods (order). Degraded organisms were identified and counted based on numbers of individual or paired characteristic structures. All recognizable prey items in the anterior section were identified, counted, and when possible, photographed using a scope-mounted digital camera (Nikon Coolpix 4500, Nikon Inc.). Prey lengths were estimated from these images using calibrated image analysis software (Image-Pro Express v4.0; Media Cybernetics 2000).

Prey that could not be placed into a species or class–order category were identified to the lowest possible taxonomic resolution. The contribution of animals within these broader categories to specific taxonomic groups was estimated by apportioning the numbers in the larger grouping to specific taxa according to the known ratios of these specific taxa for identified prey. For example, copepods that could not be identified as either cyclopoid or calanoid were placed in an unknown copepod category. After the entire sample had been counted, the proportions of cyclopoid and calanoid copepods in the identifiable sample were used to classify the unknown copepods. These estimated numbers were added to the calanoid and cyclopoid copepod counts.

All large prey items found in the posterior limb were enumerated, including *B. longimanus*, *C. pengoi*, amphipods, *M. relicta*, chironomids, and insects. Completely intact *B. longimanus*, *C. pengoi*, amphipod, and *M. relicta* individuals also were photographed. The remaining microinvertebrate fraction in the posterior limb was subsampled. Prey items in subsamples were enumerated and compared; additional aliquots were analyzed until less than 10% difference was achieved. As with the anterior gut section, intact animals in subsamples were photographed. The numbers of each prey item in the posterior limb were estimated by direct proportion. Counts from the anterior and posterior sections were combined to determine the total number of each prey item in the digestive tract.

Prey biomass was estimated by applying length – dry mass regression models, developed using net-captured invertebrates, to lengths of photographed prey items. For organisms that were not measurable because of digestion or breakage or for numbers of individuals estimated through direct proportion, average values for prey items found in fish digestive tracts or frozen net tow samples, typically from the same lake and during the same month and year, were used. In rare instances, literature values were used to estimate biomass (e.g., chironomids).

Through field correlations, Parker et al. (2001) found that the caudal process of *B. longimanus* likely is retained in the digestive tract of rainbow smelt for a longer period of time than is the rest of the invertebrate's body, with spines ultimately accumulating in the stomach. Although untested, tail spine retention also may occur following consumption of *C. pengoi*. Therefore, the use of tail spine counts alone may

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lead to overestimates of the cladocerans' contributions to fish diets. In the present study, tail spines largely were used to enumerate *B. longimanus* and *C. pengoi* numbers in the diet; following digestion, this is the most readily identifiable structure. To account for potential bias due to tail spine retention, *B. longimanus* mandible pairs also were counted in fish for several alewife ($n = 21$) and rainbow smelt ($n = 20$) caught in Lake Erie during July or October of 2002. These data then were used to develop a correction factor for tail spine retention. In these analyses, counts were combined from July (alewife, $n = 8$) and October (alewife, $n = 13$; rainbow smelt, $n = 20$) to increase sample size. It is extremely difficult to count the small *C. pengoi* mandible pairs accurately, so the *B. longimanus* correction was applied to counts of both exotic invertebrates. Tail spine retention is likely a complex process depending on gut fullness, diet, temperature, and other factors. Until experiments can provide mechanistic estimates of spine retention, all corrections (e.g., Parker et al. 2001) are based on this type of correlative gut data and may be causing substantial error in diet estimation. However, especially for *C. pengoi*, the mandibles are so small that counting these instead of tail spines likely also introduces measurement error. Because of current uncertainties in these correction factors, both corrected and uncorrected data, representing lower and upper consumption estimates, are presented to bound possible values.

Estimation of invertebrate densities in the water column

Preserved invertebrates from all 500 and 50 µm net tows were enumerated under a stereomicroscope to estimate invertebrate density in the water column (individuals·m^{-3}). Because *C. pengoi* has a tendency to clump into large aggregations during net capture (A. Storch, personal observation), all individuals in each sample were counted. Individuals were grasped by the caudal appendage and gently pulled from the clump with a pair of jeweler's forceps. All *C. pengoi* in these samples were identified to instar. All *B. longimanus* and *L. kindti* individuals found in samples also were counted. Remaining zooplankton were subsampled and additional aliquots enumerated until less than 10% difference in counts was achieved between subsamples. Subsampled organisms were identified to the lowest feasible classification (generally genus or species, except for some copepods). Counts of duplicate samples from each site (see Invertebrate collection section) were averaged to arrive at final values.

Stable carbon isotope analyses

For stable carbon isotope analyses, whenever possible, macro- and micro-invertebrates were sorted from deep-frozen samples (–80 °C) collected from each lake on sampling dates when fish also were collected. Animals generally were sorted to species, with the exception of copepods, which were sorted to order (as in the diet analysis). Invertebrate and fish muscle tissue samples were freeze-dried and weighed (approximately 200–500 µg per sample) into tin boats. Owing to their low weight, many invertebrates were pooled to make a single sample (approximately 100–400 individuals·sample^{-1}). Samples were analyzed on an elemental analyzer (Costech Analytical) coupled to a stable isotope ratio mass spectrometer (ThermoFinnigan Delta XL Plus via a ThermoFinnigan Conflo III interface). Whenever possible (when there was sufficient prey biomass), aliquots of the same sample were analyzed to evaluate measurement error. Replicate samples for each species (again, comprising multiple individuals) were used to calculate standard errors.

Stable carbon isotope values are expressed in the delta (δ) notation or in units of parts per thousand (‰) relative to Vienna Pee Dee Belemnite (VPDB) as follows:

$$(1) \qquad \delta^{13}\mathrm{C} = \left(\left[\frac{R_{\mathrm{sample}}}{R_{\mathrm{standard}}} \right] - 1 \right) \times 1000$$

where $R = {}^{13}\mathrm{C}/{}^{12}\mathrm{C}$.

A carbon isotope value closer to zero (i.e., less negative) indicates a sample contains proportionally more of the heavy stable carbon isotope (^{13}C; Peterson and Fry 1987). Lipids, such as fatty acids, are depleted in ^{13}C (DeNiro and Epstein 1977), potentially biasing isotopic values when some taxa, such as calanoid copepods, sequester more lipids than others, including many cladocerans (Arts et al. 1992). Because lipids were not extracted from all samples before stable isotope analysis, the δ^{13}C values for nonextracted invertebrate samples were corrected using taxon-specific extracted versus nonextracted sample regression models (cladoceran model: $y = 0.78x - 4.03$, $n = 28$, $r^2 = 0.91$; copepod model: $y = 0.53x - 10.65$, $n = 18$, $r^2 = 0.72$). For *Mysis relicta*, lipid-extracted data were not available to quantify this relationship. Therefore, to correct for bias due to the lipid content of these organisms, we developed a second model by regressing pooled total fatty acid contents of invertebrates and differences between extracted and nonextracted isotope signatures ($y = -0.01x - 0.84$, $n = 30$, $r^2 = 0.83$; Smyntek et al. 2007). This model was then applied to *M. relicta* total fatty acid values to estimate differences in carbon isotope ratios between extracted and nonextracted samples. In these cases, we assumed that total fatty acid content is indicative of lipid content (Olsen 1999), as the major lipid components in animals are fatty acids in the form of triacylglycerides and phospholipids.

Data analyses

Isotopic mixing

To compare the snapshot planktivore diet composition described by gut content analyses with the long-term assimilated diet represented by carbon stable isotopes, we applied linear mixing models to measured biomass diet proportions and stable isotope signatures of prey to predict stable carbon isotope ratios of planktivorous fishes. We then compared measured stable carbon isotope values of fish with these predictions based on our snapshot diet models. Static mixing models frequently are used to estimate the proportional contribution of different prey items to a consumer (Phillips and Gregg 2001). The stable carbon isotope signature of a predator, as described by the proportional diet contribution and isotopic signatures of three prey types, can be represented as follows:

$$(2) \quad \delta^{13}\mathrm{C}_{\mathrm{predator}} = A{\cdot}a + B{\cdot}b + C{\cdot}c + \mathrm{frac}$$

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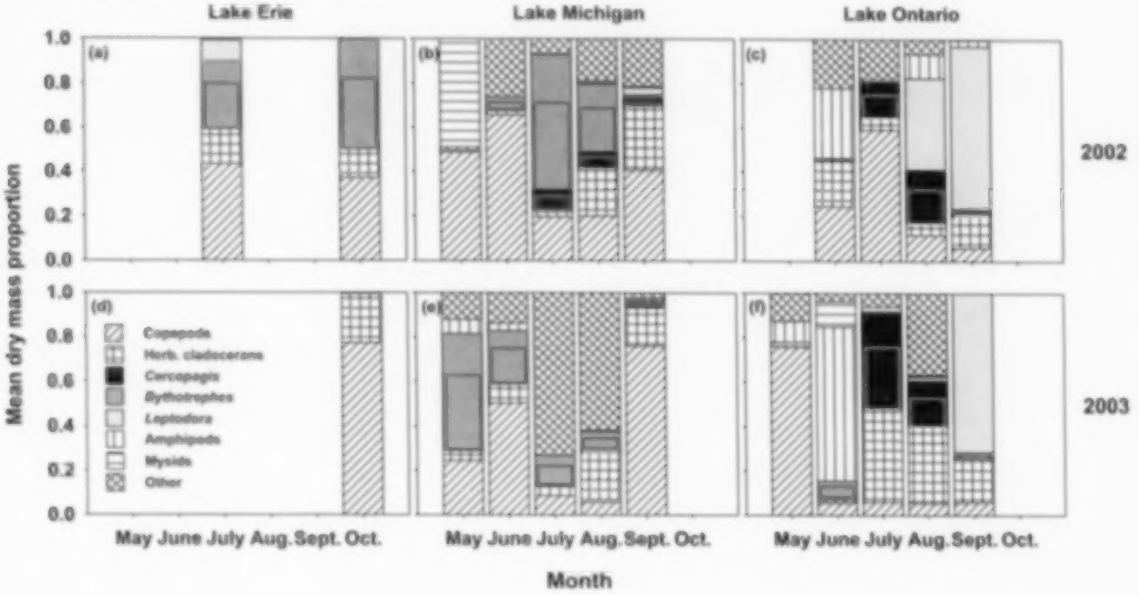
Fig. 1. Mean proportions (biomass) of prey in alewife (*Alosa pseudoharengus*) diets in eastern Lake Erie (*a*, 2002 and *d*, 2003), southwestern Lake Michigan (*b*, 2002 and *e*, 2003), and southern Lake Ontario (*c*, 2002 and *f*, 2003). Heights of the rectangles within the bars represent proportions of *Bythotrephes longimanus* (black rectangle) and *Cercopagis pengoi* (white rectangle) in the diet corrected for tail spine retention (not shown for values <1%). Herbivorous cladocerans include daphnids, bosminids, and chydorids. "Other" category includes chironomid larvae, ostracods, and aquatic dipterans. Fish were not caught in Lake Erie during July 2003 or in Lake Ontario during May 2002. See Table 2 for sample sizes.



where *A*, *B*, *C* are dry mass proportions of different prey assimilated by the predator; *a*, *b*, *c* are the respective $\delta^{13}C$ signatures of the prey; and frac is the isotopic change (‰) with increasing trophic level due to metabolic fractionation.

Isotopic mixing models typically are used to estimate diet proportions, based on known isotope signatures of predator and prey alone (without confirming traditional diet analysis), by solving for *A*, *B*, and *C* in eq. 2 ($1 = A + B + C$; $0 \leq (A, B, C) \leq 1$). In this study, fish gut content and stable carbon isotope analyses were conducted on the same organisms. Therefore, rather than solving for diet proportions based on predator and prey stable carbon isotope signatures alone, we calculated a predicted $\delta^{13}C$ value of a predator from fish gut content composition (biomass) observed directly (Figs. 1 and 2) and known $\delta^{13}C$ values of prey items measured at the time of fish capture. This procedure allowed for the incorporation of more than three prey types (Table 3). To apply this model, we assumed (*i*) an isotopic fractionation of 1‰ with each trophic level (Peterson and Fry 1987) and (*ii*) that all invertebrate prey consumed by fish in this study contribute the same proportion of carbon as dry mass to fish (Salonen et al. 1976; Makarewicz and Likens 1979). Only values from fish for which stable carbon isotope ratios were known for at least 85% of the diet taxa were included (when less than 85% of the diet was known, the model was unresponsive to changes in diet composition). Predicted stable carbon isotope signatures for fish based on snapshot diet proportions were then compared with measured values to evaluate whether stable carbon isotope values were consistent with results from the gut content analyses (i.e., that the current diet was consistent with a stable isotope ratio that would have resulted from eating those prey over a longer period of time).

Prey selection

To evaluate the potential selection of *B. longimanus* and *C. pengoi* by Great Lakes planktivores, stomach content data and water column abundances of invertebrates were input into a selectivity coefficient model (eq. 3; Vanderploeg and Scavia 1979*a*) and subsequently standardized using a relativized electivity index (eq. 4; Vanderploeg and Scavia 1979*b*).

$$(3) \qquad W_i = \frac{r_i/p_i}{\sum_i^n r_i/p_i}$$

where p_i is the relative availability of food type i in the environment, and r_i is the relative utilization of food type i in the diet.

$$(4) \qquad E_i^* = \frac{W_i - (1/n)}{W_i + (1/n)}$$

where W_i is the selectivity coefficient (see eq. 3), and n is the number of food item types.

The selectivity coefficient (W) is a variation of the Ivlev forage ratio ($E_i^* = r_i/p_i$; Ivlev 1961) normalized so the sum

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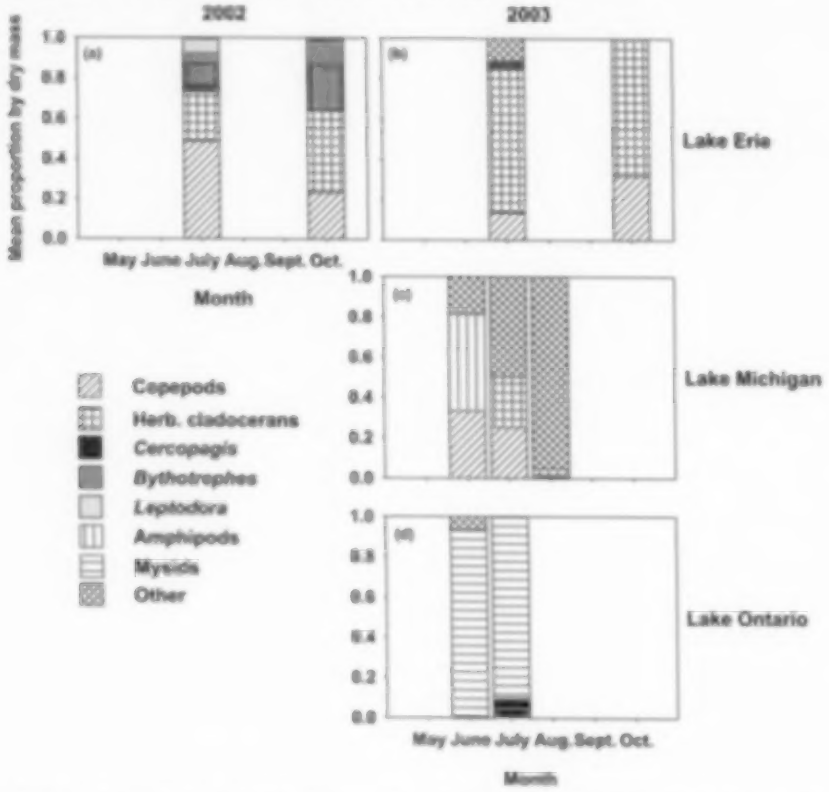
Fig. 2. Mean proportions (biomass) of prey in rainbow smelt (*Osmerus mordax*) diets in eastern Lake Erie (*a*, 2002 and *b*, 2003), southwestern Lake Michigan (*c*, 2003), and southern Lake Ontario (*d*, 2003). Heights of the rectangles within the bars represent proportions of *Bythotrephes longimanus* (black rectangle) and *Cercopagis pengoi* (white rectangle) in the diet corrected for tail spine retention (not shown for values <1%). Herbivorous cladocerans include daphnids, bosminids, and chydorids. "Other" category includes chironomid larvae, ostracods, and (or) aquatic dipterans. See Table 2 for sample sizes.



of ratios for all prey types in a sample equals one. The coefficient represents the consumer's perceived value of a food item in relation to that prey item's abundance and the abundance of other available food (Lechowicz 1982). The relativized electivity index (E^*) standardizes values of W so that predator preference is represented on a scale ranging from −1 to 1, where a value of −1 indicates complete selection against a particular prey item, 0.0 indicates that prey item is consumed in proportion to its abundance in the environment, and 1 denotes complete selection for the prey type. Fish and zooplankton were collected from the same area in each lake; however, where the planktivores were feeding vertically in the water column and whether the fish actually fed at the sampling location is not known. Thus, for electivity calculations, we assumed that alewife and rainbow smelt could exploit prey from throughout the water column and that fish consumed specifically prey from the sampling sites (i.e., feeding occurred just prior to capture or prey were distributed broadly, not patchily). These are common assumptions (e.g., Pothoven and Vanderploeg 2004), but are difficult to test in the field; as in other studies, prey and fish patchiness or movement will induce error into field electivity estimates.

Electivity coefficients were calculated by averaging the numbers of individual prey found in each fish species within a month and then calculating a single coefficient (H. Vanderploeg, personal communication). Therefore, the values indicated are composites of data from all 690 fish on which gut content analyses were performed (Table 2).

Statistical analyses

Simple linear regression was used to construct models used to estimate prey biomass and to correct for potential tail spine retention. Owing to small sample sizes, mixing model outputs were compared statistically with measured values using a Wilcoxon two-sample test. All statistical analyses were conducted using SAS statistical analysis software (SAS Institute Inc., Cary, North Carolina). Tests were considered significant at $\alpha = 0.05$.

Results

Fish gut content analysis of diet

A total of 690 fish (496 alewife and 194 smelt) were analyzed using traditional gut content analysis (Table 2). The fish were all in adult size ranges, with alewife size varying less than 41 mm (standard deviation, SD) on any sampling date. Within these narrow adult size ranges analyzed in this study, we found no discernable diet differences with body size, so no specific size-class analyses are presented.

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Table 3. Sample mixing model calculation used to predict $\delta^{13}C$ values (± standard deviation (SD)) of planktivorous fishes in Lakes Erie, Michigan, and Ontario.

Fish	Prey item	Diet proportion	$\delta^{13}C_{lipid\ uncorrected}$	$\delta^{13}C_{lipid\ corrected}$	Prey $\delta^{13}C$ contribution	$\delta^{13}C_{predicted}$	$\delta^{13}C_{measured}$
1	Calanoid copepod	0.41	−29.11±0.12	−26.20±0.06	−10.80		
	Cyclopoid copepod	0.49	−25.37±0.81[a]	−25.01±0.83[a]	−12.31		
	Bosmina	0.02	−24.67[b]	−23.16[b]	−0.52		
	Daphnia spp.	0.00	−26.53±0.59[c]	−24.49±0.43[c]	0.00		
	Cercopagis	0.06	−24.69±0.41	−23.19±0.32	−1.41		
	Bythotrephes	0.00	−25.19±0.22	−23.57±0.17	0.00		
	Harpacticoid copepod	0.01	Unknown	NA	NA	−24.04	−23.02
2	Calanoid copepod	0.00	−29.11±0.12	−26.20±0.06	0.00		
	Cyclopoid copepod	0.00	−25.37±0.81[a]	−25.01±0.83[a]	0.00		
	Bosmina	0.13	−24.67[b]	−23.16[b]	−3.11		
	Daphnia spp.	0.10	−26.53±0.59[c]	−24.49±0.43[c]	−2.42		
	Cercopagis	0.77	−24.69±0.41	−23.19±0.32	−17.78		
	Bythotrephes	0.00	−25.19±0.22	−23.57±0.17	0.00	−22.31	−24.02
3	Calanoid copepod	0.01	−29.11±0.12	−26.20±0.06	−0.25		
	Cyclopoid copepod	0.01	−25.37±0.81[a]	−25.01±0.83[a]	−0.19		
	Bosmina	0.00	−24.67[b]	−23.16[b]	0.00		
	Daphnia spp.	0.01	−26.53±0.59[c]	−24.49±0.43[c]	−0.33		
	Cercopagis	0.00	−24.69±0.41	−23.19±0.32	0.00		
	Bythotrephes	0.97	−25.19±0.22	−23.57±0.17	−22.85	−22.62	−23.47
Mean						−22.99±0.92	−23.51±0.50

Note: Predicted values were calculated using lipid-extracted stable carbon isotope ratios ($\delta^{13}C_{lipid\ corrected}$). Data are for Lake Michigan alewife (*Alosa pseudoharengus*) caught during August of 2002. Mean predicted and measured $\delta^{13}C$ values were compared for conspecific fish within months. Diet proportions represented by prey with unknown $\delta^{13}C$ signatures (i.e., harpacticoid copepods) were not included in calculations.

[a]Mean from Lake Michigan 2002 (July) and 2003 (May–September).

[b]Mean from Lake Ontario during July 2003.

[c]Mean from Lake Michigan 2002 (September) and 2003 (July–September).

Bythotrephes longimanus mandible pair counts were strongly correlated with tail spine counts in the gut contents of both alewife (slope = 0.61 ± 0.03 standard error (SE), $p < 0.0001$, $r^2 = 0.95$) and rainbow smelt (slope = 0.50 ± 0.08 SE, $p < 0.0001$, $r^2 = 0.77$) caught in Lake Erie during 2002. The slopes of models generated in this study were smaller than all but one of those estimated by Parker et al. (2001), suggesting greater tail spine retention for fish collected in this study. *Bythotrephes longimanus* and (or) *C. pengoi* were consumed more by alewife than by rainbow smelt, but were occasionally found in the diets of fish in all the lakes, at times comprising up to 70% and 46% of the alewife diet, before and after counts were corrected for tail spine retention (TSC), respectively (all diet composition values expressed in percent biomass).

Lake Erie

Alewife

Based on tail spine counts, *B. longimanus* comprised approximately 30% and 50% of the alewife diet during July and October, respectively, 2002 in Lake Erie. After TSC, *B. longimanus* biomass still accounted for approximately 20% of the diet in July and 30% in October. Copepods (calanoids, cyclopoids, and harpacticoids) also were a dominant prey item, comprising greater than 35% of biomass consumed in each month (Fig. 1*a*). The diets of alewife caught in October 2003 were dominated by copepods, and fish did not consume either of the exotic cladocerans (Fig. 1*d*).

Rainbow smelt

During July and October 2002, *B. longimanus* comprised approximately 17% (TSC = 11%) and 36% (TSC = 23%), respectively, of biomass in the diets of rainbow smelt. Small proportions of *C. pengoi* also were consumed in July of 2002 (3%; TSC = 2%; Fig. 2*a*). *Bythotrephes longimanus* was a small proportion of smelt diets in July 2003 (3%; TSC = 2%; Fig. 2*b*). During both years, the rainbow smelt diet was dominated by copepods and herbivorous cladocerans (daphnids, bosminids, and (or) chydorids; Figs. 2*a* and 2*b*).

Lake Michigan

Alewife

In Lake Michigan during May and June 2002, greater than half of the alewife diet was copepod biomass. *Bythotrephes longimanus* was consumed in June, but comprised a small proportion of the diet (5%; TSC = 3%). Both exotic cladocerans, *B. longimanus* and *C. pengoi*, were found in the gut contents during July and August; however, *B. longimanus* predominated. In July, *B. longimanus* biomass accounted for approximately 60% (TSC = 40%) of the diet, whereas *C. pengoi* comprised 9% (TSC = 6%); in August, *B. longimanus* and *C. pengoi* represented 30% and 8% (TSC = 20% and 5%) of the diet biomass, respectively. In September, copepods and herbivorous cladocerans comprised most of the diet, with less than 6% (TSC = 4%) of the diet represented by *B. longimanus* and *C. pengoi* mass (Fig. 1*b*).

In 2003, *B. longimanus* was an important diet item earlier in the season than in 2002. In May of 2003, *B. longimanus*

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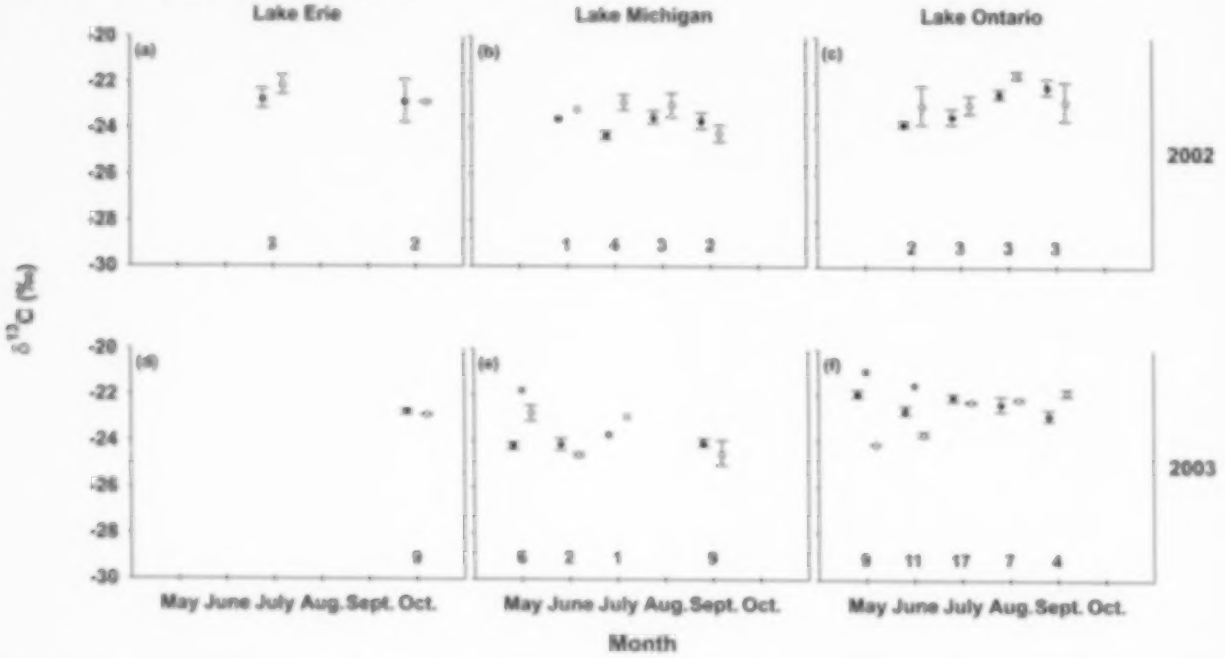
Fig. 3. Mean measured (solid circles) and predicted (open circles) stable carbon isotope signatures for alewife (*Alosa pseudoharengus*) caught in eastern Lake Erie (*a*, 2002 and *d*, 2003), southwestern Lake Michigan (*b*, 2002 and *e*, 2003), and southern Lake Ontario (*c*, 2002 and *f*, 2003). Fish were not caught in Lake Erie during July of 2003 or in Lake Ontario during May of 2002. Sample sizes, located on *x*-axis labels, are the same for measured and predicted values. Asterisks denote significant differences between paired values at $\alpha = 0.05$. Error bars are one standard error.



biomass accounted for >50% (TSC > 30‰) of gut contents. In June, the exotic cladoceran also was an important diet item (24%; TSC = 15‰); however, copepods were the dominant forage, representing over 50% of prey biomass. During July and August 2003, alewife also consumed *B. longimanus* (14%, TSC = 9‰; 8%, TSC = 5‰; respectively), but in much smaller proportions than in July and August of 2002. During July 2003, *C. pengoi* also was present in the diet, comprising less than 2% of mass consumed. In September, the diet was dominated by copepods, and *B. longimanus* represented only a small proportion of the gut content biomass (3%; TSC < 2‰; Fig. 2*e*).

Rainbow smelt

Rainbow smelt caught during 2003 in southwestern Lake Michigan did not consume either exotic invertebrate. During June, the smelt diet was composed predominantly of amphipods and copepods (48% and 33%, respectively). In July and August, rainbow smelt consumed mostly prey items within the "other" category (Fig. 2*c*).

Lake Ontario

Alewife

During June in southern Lake Ontario, alewife consumed amphipods, herbivorous cladocerans, copepods, and other prey items in equal proportions. *Cercopagis pengoi* comprised approximately 16% (TSC = 10‰) and 24% (TSC = 15‰) of the alewife diet during July and August, respectively. Further, in July, copepod biomass accounted for greater than half of alewife gut contents. In August, the native predatory cladoceran, *Leptodora kindti*, represented over 40% of alewife gut content biomass. During September, the alewife diet was dominated by *L. kindti* (Fig. 1*c*).

The diets of alewife caught during May of 2003 were composed mostly of copepod biomass (76‰). During June of the same year, alewife consumed *B. longimanus* (8‰; TSC = 5‰), but amphipods were the dominant prey item by biomass (approximately 70‰). Alewife from July and August 2003 consumed large proportions of the exotic *C. pengoi* (July: 43‰, TSC = 28‰; August: 20‰, TSC = 13‰). During these months, herbivorous cladocerans also were important diet components. During September 2003, as in 2002, the native predator *L. kindti* comprised most of the alewife gut content biomass; *B. longimanus* also was present in small proportions (3‰; TSC < 2‰; Fig. 1*f*).

Rainbow smelt

The gut contents of Lake Ontario rainbow smelt captured during June and July of 2003 were dominated largely by *Mysis relicta* (93% and 90‰, respectively); however, in July, *C. pengoi* comprised a small diet proportion (9‰; TSC = 6‰; Fig. 2*d*).

Stable carbon isotope diet comparison

No detectable differences occured between measured stable carbon isotope signatures and those predicted by a mixing model for alewife collected from Lakes Erie, Michigan, and Ontario during 2002 or from Lake Erie during 2003 (Figs. 3*a*–3*d*). However, significant differences did occur in Lakes Michigan and Ontario early in the 2003 season. In May 2003, the

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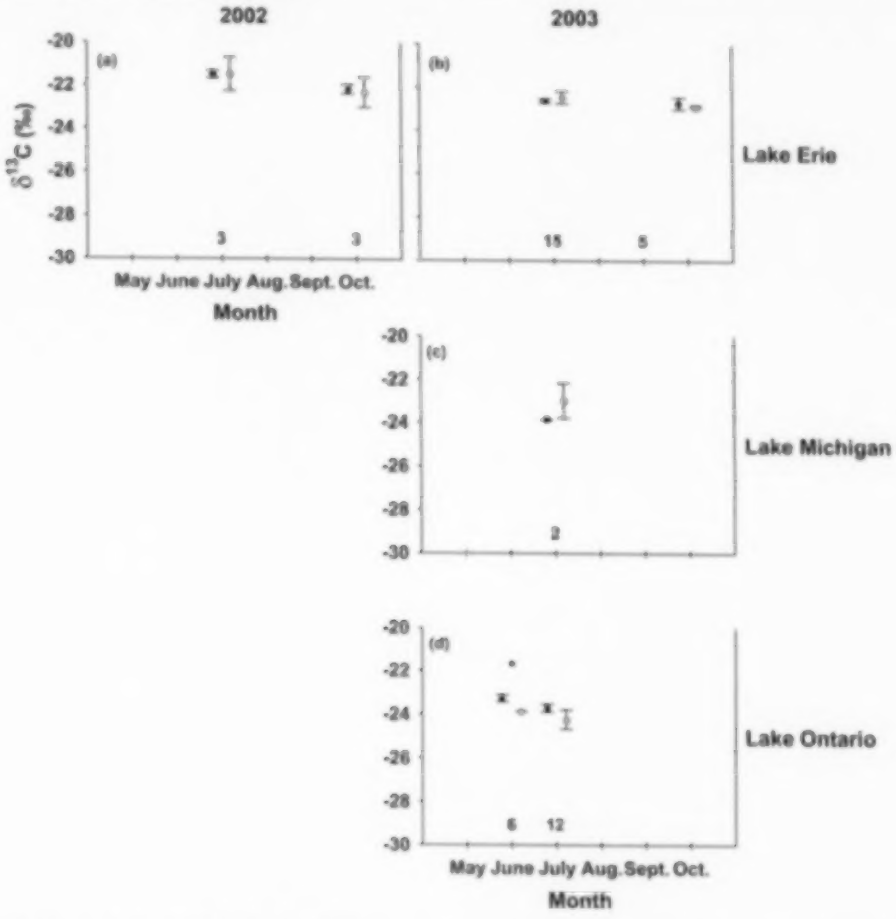
Fig. 4. Mean measured (solid circles) and predicted (open circles) stable carbon isotope signatures for rainbow smelt (*Osmerus mordax*) caught in eastern Lake Erie (*a*, 2002 and *b*, 2003), southwestern Lake Michigan (*c*, 2003), and southern Lake Ontario (*d*, 2003). Sample sizes, located on *x*-axis labels, are the same for measure and predicted values. Asterisks denote significant differences between paired values at $\alpha = 0.05$. Error bars are one standard error.



predicted isotope signature of alewife from Lake Michigan was significantly greater than the measured value ($p = 0.0453$; Fig. 3*e*). For Lake Ontario alewife collected during 2003, measured stable carbon isotopes signatures were statistically greater than predicted values during May ($p = 0.0003$) and June ($p = 0.0072$); however, no differences were found in later months (Fig. 3*f*). Measured and predicted stable carbon isotope signatures of rainbow smelt differed significantly only during June 2003 in Lake Ontario ($p = 0.0048$; Fig. 4*d*).

Prey electivities

Alewife

In general across all lakes, alewife actively selected *B. longimanus*, but *C. pengoi* was not preferred prey. When *B. longimanus* was found in fish gut contents and (or) the water column, electivity index values indicated that adult alewife in Lakes Erie, Michigan, and Ontario most often selected for this exotic (14 of 17 of sampling dates). *Bythotrephes longimanus* was never consumed in proportion to its abundance in the environment (i.e., $E^* = 0.0$) and rarely selected against (3 of 17 sampling dates). In Lake Michigan, *B. longimanus* was selected against during June of 2002 and September of 2003.

In this study, alewife commonly selected against *C. pengoi* (14 of 18 sampling dates), except in Lake Michigan during July of 2002 and 2003 and Lake Ontario during July of 2002, when the fish selected for the cladoceran (4 of 18 sampling dates). As for *B. longimanus*, alewife never consumed *C. pengoi* in proportion to its abundance in the water column (Figs. 5*a*–5*f*).

Rainbow smelt

In general across all lakes, rainbow smelt selected against both exotics. Rainbow smelt caught in the three lakes selected for and against *B. longimanus* 50% of the time, respectively. The cladoceran was never consumed in proportion to its abundance in the environment. The same held true for *C. pengoi*. In Lake Erie, rainbow smelt selected for *B. longimanus* during both months sampled in 2002 and selected against the cladoceran during July of 2003 (Figs. 6*a* and 6*b*). *Bythotrephes longimanus* was selected against dur-

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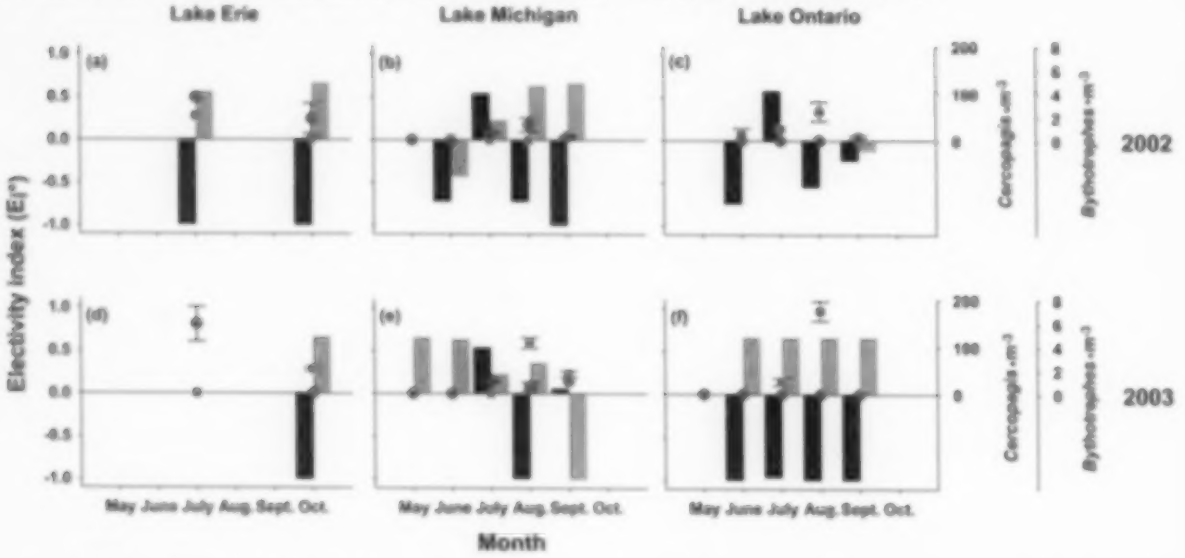
Fig. 5. Electivity index values for alewife (*Alosa pseudoharengus*) caught in eastern Lake Erie (*a*, 2002 and *d*, 2003), southwestern Lake Michigan (*b*, 2002 and *e*, 2003), and southern Lake Ontario (*c*, 2002 and *f*, 2003). Negative values indicate that a prey item was selected against; positive values show selection for a prey item. Electivities were calculated from the mean (see Table 2 for sample sizes) occurrence of prey in gut contents during the specified sampling period. Values were not calculated for months when a prey item was absent in both gut contents and the environment. Bars represent electivity index values for *Cercopagis pengoi* (solid) and *Bythotrephes longimanus* (shaded). Solid circles show abundances of *C. pengoi* (black with white cross-hairs) and *B. longimanus* (gray with black cross-hairs) per cubic metre in the water column. Error bars are one standard error. Note different scales on abundance axes.



ing June and July 2003 in Lake Michigan and selected for during June in Lake Ontario (Figs. 6*c* and 6*d*). Lake Erie rainbow smelt selected against *C. pengoi* during July and October of 2002. In 2003, the cladoceran was selected for during July and selected against in October (Figs. 6*a* and 6*b*). Rainbow smelt caught in both Lakes Michigan and Ontario during 2003 selected for *C. pengoi* (Figs. 6*c* and 6*d*).

Discussion

The importance of the exotic crustaceans *B. longimanus* and *C. pengoi* in the diets of Great Lakes planktivores has been studied in individual systems (e.g., Mills et al. 1992; Parker et al. 2001; Bushnoe et al. 2003), but to our knowledge no studies have compared directly the contributions of these invertebrates to fish diets in different water bodies. Although our spatial coverage on each lake was constrained because of cost and logistics to a few sites, and most fish were collected in nearshore areas, sometimes with varying gear, our collection of monthly data at each site over two growing seasons did allow us to identify some generalizable patterns. Our results show that one or both of the exotics are seasonally important prey for adult alewife in several of the Great Lakes (Lakes Erie, Michigan, and Ontario; up to 70% of the diet) and occasionally for adult rainbow smelt in Lake Erie (up to 35% of the diet). Further, consumption of *B. longimanus* and (or) *C. pengoi* varied among lakes and seasonally, apparently because of differential abundances of the exotics.

In Lake Ontario, *C. pengoi* comprised large proportions of the alewife gut content biomass during July and August of 2002 and 2003, but *B. longimanus* was consumed rarely. The absence of *B. longimanus* in the diets of Lake Ontario alewife likely was due to low availability during the study years. Low abundances of *B. longimanus* in Lake Ontario may result from lack of suitable or attainable prey. When offered a choice, *B. longimanus* consumed medium to large *Daphnia pulicaria* in the laboratory (Schulz and Yurista 1999). This exotic cladoceran preferred slower moving *D. pulicaria* over calanoid copepods. At sampling sites in Lake Ontario, copepods dominated, whereas *Daphnia* spp. were less abundant. Further, analyses of zooplankton lengths showed that Lake Ontario *Daphnia* spp. typically were smaller than those found in Lakes Erie and Michigan (K.L. Schulz, unpublished data). Benoit et al. (2002) found evidence indicating that *C. pengoi* likely feed on the smaller zooplankton fraction, including copepod nauplii. In Lake Ontario, juvenile copepods may help to sustain *C. pengoi* populations, making the exotic abundant and important prey for alewife. Larger contributions of *B. longimanus* (compared with *C. pengoi*) to alewife diets in Lakes Erie and Michigan may be due either to selection for the large cladoceran (see below) or greater availability in the water column. Regardless of underlying mechanisms, this study shows one or both of the exotic cladocerans *B. longimanus* and *C. pengoi* are important prey for adult alewife in Lakes Erie, Michigan, and Ontario during the growing season.

Unlike alewife, rainbow smelt analyzed in this study generally consumed few or no *B. longimanus* or *C. pengoi*. These findings are consistent with Bushnoe et al. (2003) for rainbow smelt in Lake Ontario. Rainbow smelt sampled in our study typically were smaller than the alewife. Because

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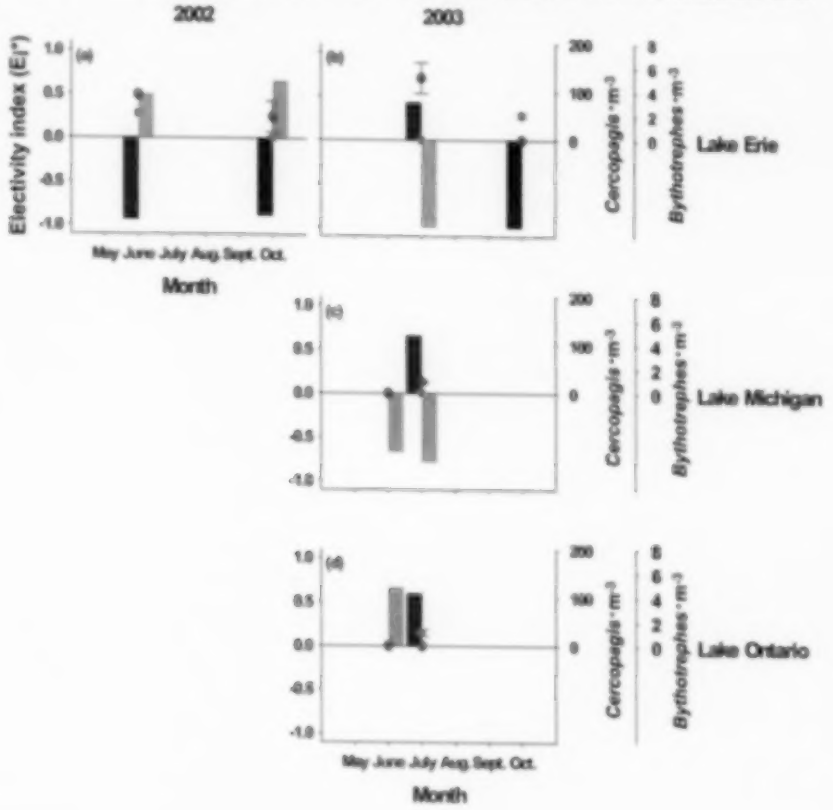
Fig. 6. Electivity index values for rainbow smelt (*Osmerus mordax*) caught in eastern Lake Erie (*a*, 2002 and *b*, 2003), southwestern Lake Michigan (*c*, 2003), and southern Lake Ontario (*d*, 2003). Negative values indicate that a prey item was selected against; positive values show selection for a prey item. Electivities were calculated from the mean (see Table 2 for sample sizes) occurrence of prey in gut contents during the specified sampling period. Values were not calculated for months when a prey item was absent in both gut contents and the environment. Bars represent electivity index values for *Cercopagis pengoi* (solid) and *Bythotrephes longimanus* (shaded). Solid circles show abundances of *C. pengoi* (black with white cross-hairs) and *B. longimanus* (gray with black cross-hairs) per cubic metre in the water column. Error bars are one standard error. Note different scales on abundance axes.



both exotic zooplankton have long chitinous tail spines, gape limitations may prevent small fish from consuming *B. longimanus* and *C. pengoi* (e.g., Barnhisel and Harvey 1995; Vanderploeg et al. 2002). However, rainbow smelt larger than 70 mm can consume both *B. longimanus* and *C. pengoi* (Barnhisel and Harvey 1995; Bushnoe et al. 2003). Therefore, gape limitation likely was not a factor contributing to the general absence of the exotics in rainbow smelt diets in this study. In the Great Lakes, rainbow smelt consume large quantities of macroinvertebrates, such as *Mysis relicta*, relying less on smaller zooplankton (Urban and Brandt 1993; Johnson et al. 1998). Further, rainbow smelt in Lake Erie exploited ephemeral prey, such as chironomid pupae (Parker-Stetter et al. 2005). Based on our data and these previous studies, these exotics may be generally less important for rainbow smelt during the growing season than they are for alewife. The small quantities of *B. longimanus* and *C. pengoi* consumed by rainbow smelt, compared with alewife, likely reflect general resource partitioning between the two fish species, perhaps resulting from longer periods of vertical overlap between the exotics and alewife during the growing season.

Based on field correlations, Parker et al. (2001) concluded that the chitinous tail spine of *B. longimanus* is retained in smelt digestive tracts longer than the organism's body, and the use of tail spine counts to enumerate the cladoceran in the diet may lead to overestimates. To correct for potential bias due to *B. longimanus* and *C. pengoi* tail spine retention, we applied models relating *B. longimanus* tail spine and mandible-pair counts. Even with corrected biomass proportions, *B. longimanus* comprised up to 40% of the gut content biomass and *C. pengoi* as much as 28%.

The slopes of our models were smaller than most estimated by Parker et al. (2001), indicating greater tail spine retention by fish used to quantify the relationships in our study. Therefore, our estimates of *B. longimanus* and *C. pengoi* consumption by fish after accounting for differential digestion likely are conservative. Further, *C. pengoi* caudal appendages are generally more flexible (Snyder 2004; A. Storch, personal observation) than those of *B. longimanus*

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and may pass through fish digestive tracts more easily. Therefore, use of *B. longimanus* mandible – tail spine relationships may not be appropriate to correct for the retention of *C. pengoi* tail spines, but again, this is almost certainly a conservative measure. Future research should test experimentally for the differential digestion of *B. longimanus* and *C. pengoi* to evaluate and further reduce potential bias in fish gut content enumeration.

Traditional gut content analyses provide a description of specific prey taxa most recently consumed, whereas stable carbon isotope analyses describe the time-integrated diet. Most studies rely on only one of these types of analyses to determine diet. Used in conjunction, these analyses illustrate specific prey compositions in the diet that are assimilated over a period of time. We used a static mixing model to estimate the stable carbon isotope signatures of alewife and rainbow smelt, based on observed fish diet compositions and stable carbon isotope values of prey, to verify that the gut content analysis snapshots are consistent with longer-term diet. To our knowledge, simultaneous gut content and stable isotope analyses have not been done to evaluate the roles of exotic species in the diets of consumers. Comparisons between observed adult fish gut contents and stable carbon isotope values of prey can be problematic because of slow rates of tissue turnover (e.g., Hesslein et al. 1993; Vander Zanden et al. 1998). The agreement of all but three of our monthly predictions from snapshot data with the observed stable isotope data is consistent with relatively fast tissue turnover and relatively constant diet over a several week time frame (i.e., the warm growing season). The 3 months when we found significant differences between measured stable carbon isotope signatures of fish muscle tissue and those values predicted from the diet were all in late spring – early summer. These differences likely resulted from stable carbon isotope signatures of muscle tissue still reflecting the early spring diet and colder water temperatures leading to increased tissue turnover times, lengthening the period required for the fish to reflect the isotope signatures of a changed diet. During late summer and early fall, at times of warmer temperatures, the stable carbon isotope and diet data were consistent. Taken together, both stable carbon isotope and gut content analyses suggest that alewife, and to a lesser extent rainbow smelt, can consume and assimilate large quantities of the exotic cladocerans *B. longimanus* and *C. pengoi*.

As visual predators, fish often select large and easily attainable prey items to optimize foraging efficiency. Alewife, and less commonly rainbow smelt, often actively selected the larger exotic cladoceran *B. longimanus* when it was present in the diet. These results are consistent with the findings of Pothoven and Vanderploeg (2004) and reflect size-biased feeding and optimal foraging theory (Gerking 1994). Conversely, *C. pengoi* in the water column largely was selected against. Although typically over an order of magnitude lighter than *B. longimanus*, *C. pengoi* is similar in length to *B. longimanus*, including its caudal appendages (A. Storch, personal observation). Therefore, like *B. longimanus*, one would expect *C. pengoi* to be a preferred prey item. However, we generally found selection against *C. pengoi*. At the sampling sites in Lakes Erie, Michigan, and Ontario, *C. pengoi* was found in much greater abundance than *B. longimanus*. Owing to the high abundance of *C. pengoi* in the water column, fish consumed large quantities of the cladoceran even though they did not prefer it over other prey.

As *B. longimanus* and *C. pengoi* spread throughout North America, an understanding of their specific consumption by planktivores and their roles in fish diets becomes increasingly important. Whereas the tail spine of *B. longimanus* likely is retained in fish digestive tracts, potentially requiring added energy expenditure and impacting fish condition (Parker et al. 2001), *C. pengoi* may pass more readily. Further, the nutritional quality of *B. longimanus* and *C. pengoi* may differ. Therefore, fish may be differentially impacted by the consumption of *B. longimanus* versus *C. pengoi*. Alewife consume large quantities of the exotics, whereas the diets of rainbow smelt contained small proportions. Further, other planktivores, such as yellow perch (*Perca flavescens*), may shred the exotics after consumption (A. Storch, personal observation). Thus, the effects of *B. longimanus* and *C. pengoi* invasions likely also differ among fish species.

This study highlights the importance of the exotic zooplankton *B. longimanus* and *C. pengoi* in the diets of alewife and occasionally rainbow smelt in several Great Lakes, especially in the nearshore zone. One or both of these exotic cladocerans, even when not actively selected for, can be abundant prey in the diets of Great Lakes planktivores throughout the summer months. These findings may have important implications for the condition and production of Great Lakes zooplanktivores, especially alewife. Parker et al. (2001) indicated that the caudal appendages of *B. longimanus* may accumulate in rainbow smelt digestive tracts, having potentially negative consequences on fish condition by reducing feeding rates. Further, Parker-Stetter et al. (2005) suggested that accumulation of tail spines may adversely impact seasonal growth of rainbow smelt by reducing realized daily rations. Because planktivores, such as alewife and rainbow smelt, are primary prey for economically important salmonines in the Great Lakes (Mills et al. 1993; Madenjian et al. 2003; Mills et al. 2003), impacts on prey fish populations resulting from consumption of *B. longimanus* and (or) *C. pengoi* eventually may reach higher trophic levels.

Acknowledgements

We thank Karin Limburg, Edward Mills, two anonymous reviewers, and editor W. Gary Sprules for helpful comments on early drafts of this manuscript. We are also grateful to Donald Einhouse and the crew of the R/V *Argo*, Christopher Hotaling, Jeff Jones, Allison Witt, Elaine Langer, Theodore Lewis, Joe Makarewicz, and Tim Mihuc for providing helpful insight and assistance. This document is a resulting product from project R/CMB-27, funded under award NA16RG1645 (to K.L. Schulz and C.E. Cáceres) from the National Sea Grant College Program of the US Department of Commerce's National Oceanic and Atmospheric Administration, to the Research Foundation of State University of New York on Behalf of New York Sea Grant. These statements, findings, conclusions, views, and recommendations are those of the authors and do not necessarily reflect the views of any of those organizations. Supplemental funding for this research was awarded to A.J. Storch by New York State Sea Grant (Thesis Completion Award), The Edna Bailey Sussman Foundation, and the Sigma Xi Grants in Aid of Research program, and

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additional funding was provided by the National Science Foundation under grant No. 0416308.

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Predicting the invasion success of an introduced omnivore in a large, heterogeneous reservoir

Shane Vatland and Phaedra Budy

Abstract: We demonstrate that invasion success, through the introduction and establishment stages, can generally be predicted based on biological characteristics of the organisms and physical aspects of the environment; however, predicting subsequent effects during integration is more challenging, especially for omnivorous fish species in large, heterogeneous systems. When gizzard shad (*Dorosoma cepedianum*) were incidentally introduced into Lake Powell, Utah–Arizona (2000), we predicted they would be successful invaders and would have food-web effects ranging from neutral to negative. As predicted, gizzard shad successfully established and dispersed throughout this large reservoir (300 km) within just 4 years, and their density was positively correlated with productivity. Also as predicted, gizzard shad exhibited fast growth rates, and striped bass (*Morone saxatilis*) predators were thus gape-limited, obtaining little gizzard shad forage. Contrary to our predictions, however, competition over zooplankton resources between gizzard shad and both threadfin shad (*Dorosoma petenense*) and juvenile striped bass appeared limited because of spatial segregation and diet preference. In sum, gizzard shad will continue to be successful invaders, but with limited effects on the established predator–prey cycle.

Résumé : Nous démontrons que le succès d'une invasion, du moment de l'introduction à celui de l'établissement, peut généralement être prédit d'après les caractéristiques biologiques des organismes et les caractéristiques physiques du milieu; cependant, la prédiction des effets subséquents durant l'intégration est un défi plus important, particulièrement chez les espèces de poissons omnivores, dans les grands systèmes hétérogènes. Lorsque des aloses à gésier (*Dorosoma cepedianum*) ont été introduites fortuitement au lac Powell Utah–Arizona en 2000, nous avons prédit que l'invasion serait réussie et que les effets sur le réseau alimentaire varieraient de neutres à négatifs. Comme prédit, les aloses à gésier se sont établies avec succès et se sont dispersées dans l'ensemble de ce grand réservoir (300 km) en seulement quatre années; leur densité était en corrélation positive avec la productivité. Aussi comme prévu, les aloses à gésier ont connu des taux de croissance élevés, mais les bars rayés (*Morone saxatilis*), des prédateurs limités par l'ouverture de leur bouche, ont peu utilisé les aloses à gésier comme poissons fourrage. Cependant, contrairement à nos prédictions, la compétition pour les ressources du zooplancton entre les aloses à gésier et à la fois les aloses fils (*Dorosoma petenense*) et les jeunes bars semble limitée à cause de la ségrégation spatiale et des préférences alimentaires. En somme, les aloses à gésier continueront d'être de bons envahisseurs, mais avec des effets limités sur le cycle établi des prédateurs et des proies.

[Traduit par la Rédaction]

Introduction

While most invasive species fail soon after introduction (Williamson and Fitter 1996), successful invasions have significant ecological and economical impacts at a global scale (Vitousek et al. 1997; Mack et al. 2000; Pimentel et al. 2000). Further, the unplanned nature of most invasions often results in the subsequent lack of quantitative data collected at an appropriate spatial scale (e.g., Moyle and Light 1996*a*) and presents challenges for predicting the ecological impact of invasive species in the future (Ruiz and Carlton 2003). The role of fish invaders in large, lentic systems can be especially challenging to unravel because of the inherent complexities of multitrophic-level food webs (e.g., Vander Zanden et al. 1999), common shifts in habitat use exhibited by many fish species (e.g., Garvey et al. 1998), difficulties in separating the relative roles of endogenous effects from exogenous forces (e.g., Ives et al. 1999), and potentially mismatched evolutionary histories (e.g., Gido et al. 2000); these factors may act independently or in combination to obscure the role of an invader within an aquatic food web.

Despite these challenges, there has been considerable effort and progress toward describing the suite of characteristics that determine the success and potential effects of invasive fish species (Ruiz and Carlton 2003; Moyle and Marchetti 2006). While there are several very general char-

Received 21 July 2006. Accepted 4 June 2007. Published on the NRC Research Press Web site at cjfas.nrc.ca on 26 September 2007. J19437

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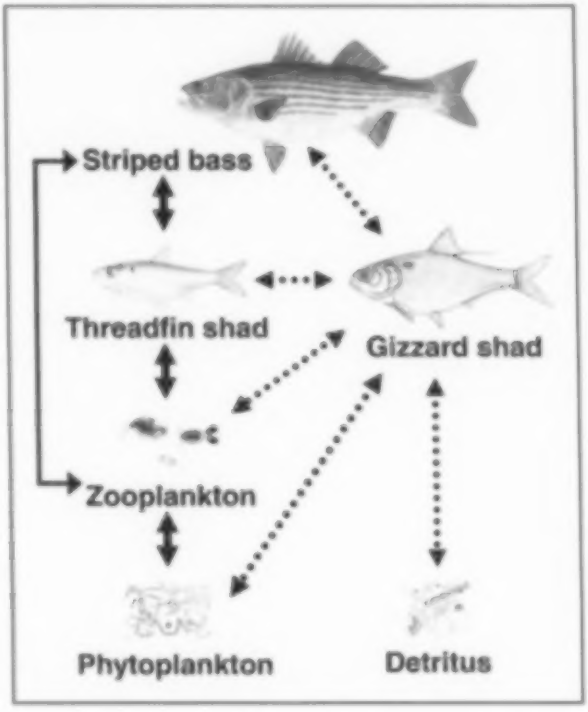
 doi:10.1139/F07-100 © 2007 NRC Canada

acteristics of successful fish invaders, including the ability to reproduce rapidly (Lodge 1993), having a broad native range (Ricciardi and Rasmussen 1998), and being a habitat and (or) diet generalist or omnivore (Moyle and Light 1996*a*, 1996*b*), consideration of the influence of different factors at specific stages of invasions has been revealing. For example, Fuller (2003) suggested the establishment of invasive fish is largely dependent on attributes of the introduction pathway (e.g., number of fish introduced and frequency of introduction); Marchetti et al. (2004*a*) considered three stages (establishment, spread, integration) of the invasion process and evaluated the relative importance of factors including trophic status, size of native range, propagule pressure, parental care, maximum adult size, physiological tolerance, distance from nearest native source, and prior invasion success. Even with these advances, however, there is still a need to (*i*) better understand the relative influence and consistency of these different factors across the stages of invasion and (*ii*) gain a better understanding of the invasion process in both natural systems and predominantly human-altered waterways (Havel et al. 2005). This later includes the importance of evaluating the role of established alien species in determining the outcome of a new invasion (Moyle and Marchetti 2006). Collectively, these studies provide a general framework for assessing the potential effects of invasive fish species across a broad range of both natural and highly altered aquatic ecosystems (Kolar and Lodge 2001; Peterson et al. 2004; Olden et al. 2006).

Gizzard shad (*Dorosoma cepedianum*), an opportunistic omnivore, are frequently introduced (intentionally and unintentionally) as forage for piscivorous sport fish in freshwater lakes and reservoirs. These introductions have resulted in highly variable, but sometimes predictable, effects on aquatic ecosystems (Bremigan and Stein 2001; Vanni et al. 2005). Introduced gizzard shad are capable of dominating food-web dynamics from an intermediate trophic position (Stein et al. 1995); this dominance is attributed to a combination of gizzard shad life-history characteristics and positive feedback loops within the invaded food webs (DeVries and Stein 1992; Michaletz 1998*a*; Schaus and Vanni 2000). Conversely, introduced gizzard shad may be limited by winter die-offs in north-temperate systems (Stein et al. 1995), density-dependent growth and reproduction (Allen et al. 2000; Clayton and Maceina 2002), and, subsequently, piscivore control (Vanni et al. 2005). However, the conditions under which piscivores can control gizzard shad populations are not implicitly understood (Dettmers et al. 1998; Irwin et al. 2003).

In 2000, gizzard shad were discovered in Lake Powell, a large, oligotrophic reservoir on the Utah–Arizona (USA) border, offering a unique opportunity to gain a better understanding of the potential effects of an invasive omnivore on a large freshwater ecosystem. Although Lake Powell represents a highly altered artificial system with a species assemblage determined solely by both incidental and purposeful introductions, we believe this opportunity presented a rare opportunity to learn, as (*i*) we initiated our studies near the point (time and space) of initial invasion and comprehensively followed this invasion through all stages, and (*ii*) Lake Powell's dominant pelagic fish species, striped bass (*Morone saxatilis*) and threadfin shad (*Dorosoma petenense*), have been relatively well-studied and exhibit a strong 5- to 7-year predator–prey cycle (Vatland 2006), offering an opportunity to explore the effects of an invader on an established predator–prey cycle. The potential effects of gizzard shad on Lake Powell's aquatic food web (Fig. 1) are a major concern, as the reservoir supports an extremely popular inland striped bass fishery, and the unique, endemic fishes of the Colorado River remaining in sections above and below the reservoir are already imperiled by a diverse assemblage of other exotic fishes and nuisance species (Olden et al. 2006; Stokstad 2007).

Fig. 1. Simplified, conceptual diagram of the aquatic food web of Lake Powell with established food-web interactions (solid lines) and potential gizzard shad (*Dorosoma cepedianum*) interactions (broken lines). Diagram is not drawn to scale. In 2003–2005, the observed maximum size (measured in total length) of threadfin shad (*Dorosoma petenense*), gizzard shad, and striped bass (*Morone saxatilis*) was 187, 551, and 1162 mm, respectively.



We used this unique opportunity in Lake Powell to develop, and then test, a set of a priori hypotheses of invasion success and food-web effects based on generalities available in the literature, the physical environment, and our own ecological understanding of the invaded ecosystem. Based on general characteristics of gizzard shad, such as high fecundity, omnivory, and prior invasion success, and tolerable winter conditions, we predicted gizzard shad would be successful invaders in Lake Powell (introduction stage). We further hypothesized the following during the different stages of invasion: (H1) the more productive inflow areas would support the highest density of gizzard shad (establishment stage); (H2) gizzard shad, especially age-0 fish, would compete with threadfin shad and juvenile striped bass for zoo-

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plankton resources (integration stage); (H3) the gizzard shad population would eventually be dominated by large, fast-growing adults, as is typical of low to moderately productive systems (integration stage); and (H4) as a result of H3, gizzard shad would provide limited forage for striped bass, and striped bass would not be capable of controlling gizzard shad through predation (integration stage). We monitored the invading gizzard shad population from near its initial introduction, through these three stages, to test our predictions and investigate the effects of the invasion on an established food web and predator–prey cycle. Our approach combined field observations of fish demographic and population information, laboratory analyses of biological factors at multiple trophic levels, and bioenergetics-based modeling of piscivore growth.

Materials and methods

Study site

Glen Canyon Dam impounds the Colorado River approximately 10 km south of the Utah–Arizona border and forms Lake Powell, one of the largest reservoirs in the United States (approximately 300 km in length, maximum depth of 178 m, and 32.1 km^3 in volume at full pool). Lake Powell is a warm, meromictic reservoir, and thermal stratification of the mixolimnion generally begins in April and persists through November. Average, daily surface water temperatures range from 6.7 to 29.4 °C (Gustaveson 1999), and mid-summer surface temperatures are typically around 25 °C (Sollberger et al. 1989). Since the reservoir reached full pool in 1980, surface elevation has fluctuated (1091–1123 m); most of the shoreline is composed of steep sandstone cliffs and talus slopes.

Lake Powell is an oligotrophic reservoir (Paulson and Baker 1983; Potter and Drake 1989) with relatively low nutrient levels; main channel chlorophyll *a* concentrations are generally less than 2 $\mu g \cdot L^{-1}$ during summer and autumn. Cladoceran zooplankton (family Cladocera) generally dominate lake-wide zooplankton biomass in the spring and summer, whereas copepod zooplankton (order Copepoda) usually dominate biomass in the autumn. Main channel macrozooplankton densities are generally fewer than 20 individuals·L^{-1} (Sollberger et al. 1989), and the distribution of zooplankton is considered to be relatively patchy (Mueller and Horn 1999). The pelagic fish community of Lake Powell is dominated by threadfin shad and striped bass. Other piscivorous fish species found in the reservoir include smallmouth bass (*Micropterus dolomieu*), largemouth bass (*Micropterus salmoides*), walleye (*Sander vitreus*), channel catfish (*Ictalurus punctatus*), and yellow bullhead (*Ameiurus natalis*); forage species, aside from threadfin shad and gizzard shad, include bluegill (*Lepomis macrochirus*), green sunfish (*Lepomis cyanellus*), black crappie (*Pomoxis nigromaculatus*), white crappie (*Pomoxis annularis*), red shiner (*Cyprinella lutrensis*), and common carp (*Cyprinus carpio*).

We collected field data in Lake Powell in the spring (May–June), summer (July–August), and autumn (October–November) of 2003, 2004, and 2005 at six index areas of the reservoir (Wahweap Bay, Padre Bay (2003 only), San Juan Arm, Bullfrog Bay, Good Hope Bay, and Trachyte Canyon; Fig. 2). We chose these areas to capture the trophic gradient of the reservoir (e.g., primary productivity decreases as distance from an inflow increases) and match sites historically sampled by the Utah Division of Wildlife Resources.

Introduction and establishment stages

We used a combination of fish sampling and limnological analyses to track the gizzard shad invasion through the introduction and establishment stages and to provide data to test our hypotheses.

Limnological analysis

To assess relationships between fish species and biota at lower trophic levels (i.e., zooplankton and phytoplankton) as well as to assess physical attributes of the reservoir (i.e., temperature and dissolved oxygen), we collected limnological data at designated, open-water sites during sample events. We collected zooplankton from depths of 8 m and approximately 40 m with vertical tows of an 80 µm mesh Wisconsin-style zooplankton net. In the laboratory, we enumerated zooplankton to genera for cladocerans and to order for copepods and measured total length (nearest 0.01 mm, from the top of the head to the base of the tail spine) on up to 30 specimens per taxa per sample. Finally, we calculated total zooplankton length distributions separately for each tow depth and subsequently weighted the proportion of zooplankton in each length class by tow depth to calculate a reservoir-wide size distribution of zooplankton. We sampled epilimnetic phytoplankton using an integrated tube sampler to collect water from a depth of 8 m; we then used the concentration of chlorophyll *a* ($\mu g \cdot L^{-1}$) as an index of phytoplankton biomass (Welschmeyer fluorometric method; Welschmeyer 1994). We also measured vertical profiles of temperature (°C) and dissolved oxygen ($mg \cdot L^{-1}$) to a depth of approximately 40 m using a temperature – dissolved oxygen meter.

Fish sampling

We used a combination of horizontal gillnetting and midwater trawling to evaluate the temporal and spatial distribution, relative abundance, and size structure (and other demographic information) of target fish in the reservoir. To sample the littoral zone and pelagic–littoral interface, we set sinking, experimental gill nets (1.8 m deep × 30 m long, four 7.6 m panels of 19, 25, 38, 50 mm bar mesh) just offshore, spanning 0–20 m depth, before dusk and pulled them at dawn, spanning two crepuscular periods. We utilized gill net catch per unit effort (CPUE) as an index of relative biomass (kilograms per net per night) and an index of relative abundance (number per net per night). To supplement gill net data and sample the pelagic zone, we conducted epilimnetic (0–12 m), midwater trawling (3 m wide × 7 m tall × 18 m long trawl with stretch mesh ranging from 102 mm near the opening to 3 mm at the cod end) at night around new moon events (Gustaveson et al. 1980; Rieman 1992). We also collected fish information from annual electrofishing surveys (September), gill net surveys (October–November), and midwater trawling (July–August) performed by the Utah Division of Wildlife Resources; multiple capture techniques minimized biases in size selectivity (Boxrucker et al. 1995).

To further test H1, we then regressed the relative abundance of gizzard shad (number per net night; independent variable) against mean chlorophyll *a* for each site and for the

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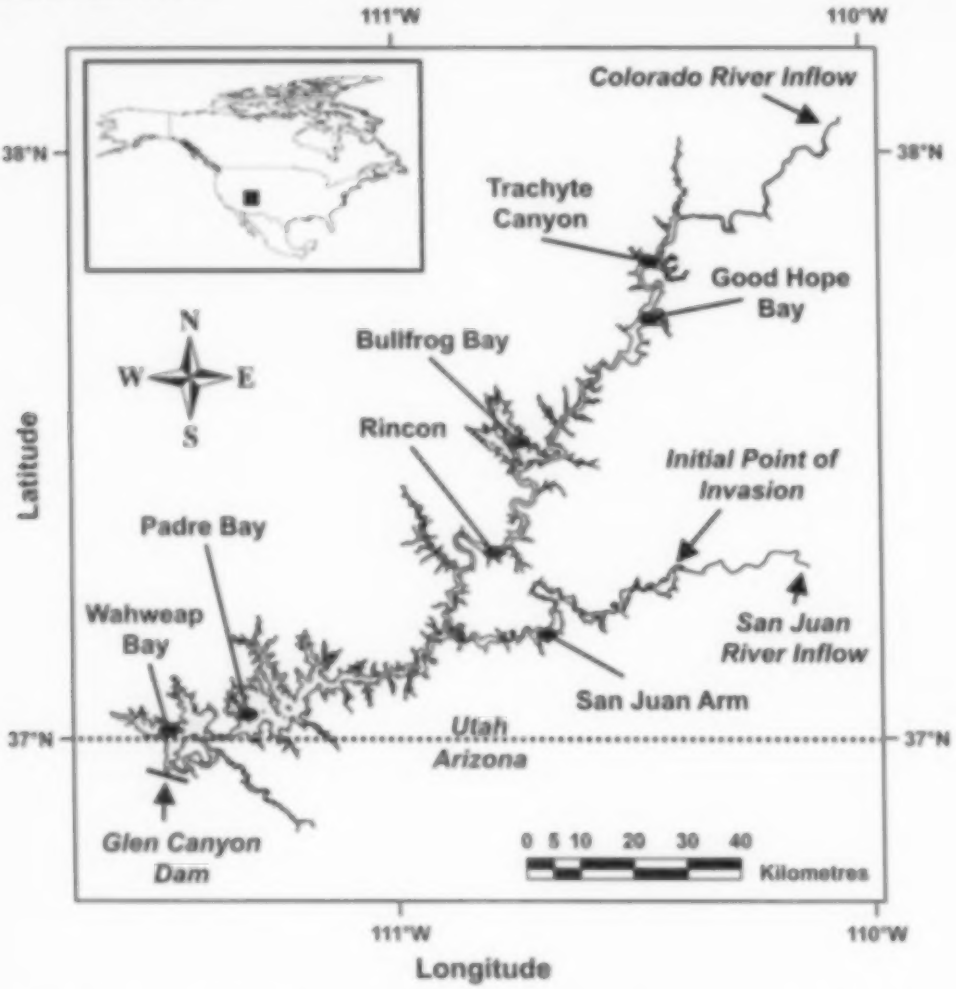
Fig. 2. Site map indicating sample areas included in this study, major inflows, and the initial point of gizzard shad (*Dorosoma cepedianum*) invasion in Lake Powell.



pelagic and littoral zones independently; we evaluated model fit and significance using R^2 and P values ($\alpha = 0.05$).

Integration stage

Zooplankton resource competition (H2)

We examined the potential for gizzard shad to compete with other fish species, primarily threadfin shad and juvenile striped bass, for food resources using quantitative diet evaluation coupled with stable isotope analysis. We collected stomachs from all gizzard shad, threadfin shad, and striped bass caught in gill net sets and quantified the gut contents by wet weight in the laboratory. When possible, prey items were identified to species for fish prey and to genus for zooplankton prey, and the length of all diet items (up to 30 individual zooplankton per stomach) was measured. If necessary, vertebral or backbone length measurements of prey fish were converted to total length using relationships developed by Raborn et al. (2002). We used this prey length information to compare the relationship among prey sizes consumed, prey sizes available in the environment, and predator gape limits.

To quantify interspecific diet overlap between both shad species and juvenile striped bass, we calculated Schoener's index (Schoener 1970) and Horn's index (Horn 1966). Indices of diet overlap are potentially biased (e.g., Smith and Zaret 1982), but both indices provided similar patterns in overlap in this study. Thus, hereafter, we present only the results of the Schoener's index:

$$D = 100\left(1 - 0.5\sum_{i=1}^{n}\left|p_{x,i} - p_{y,i}\right|\right) \qquad i = 1, 2, \ldots, n$$

where n is the number of diet categories, $p_{x,i}$ is the proportion of diet category i consumed by predator x, and $p_{y,i}$ is the proportion of diet item i consumed by predator y. We considered $D > 60$ to express a significant overlap (Wallace 1981).

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Table 1. Percentage and direction of change to input parameters used in bioenergetics-based modeling of striped bass (*Morone saxatilis*) growth.

	% of shad in striped bass diets			
Scenario	Spring	Summer	Autumn	Total consumption by striped bass
2004	NA	NA	NA	NA
E1	▲ 25%–50%	NA	NA	NA
E2	▲ 25%–50%	▲ 25%–50%	▲ 25%–50%	NA
E3	▲ 25%–50%	▲ 25%–50%	▲ 25%–50%	▲ 10%–20%
D1	▲ 25%–50%	NA	NA	▼ 10%–20%
D2	▲ 25%–50%	▼ 25%–50%	▼ 25%–50%	NA
D3	NA	▼ 25%–50%	▼ 25%–50%	NA
D4	NA	▼ 25%–50%	▼ 25%–50%	▼ 10%–20%

Note: First and second values, separated by a dash, represent changes to juvenile and adult parameters, respectively. Scenarios starting in E and D signify "enhancement" and "detriment" scenarios, respectively; NA denotes parameters that were not adjusted from the 2004 model. Symbols represent the direction of proportional change.

To further evaluate trophic position and diet overlap, we used stable isotope properties. We collected muscle tissue from above the rib cage on a subset (10 per species per area) of gizzard shad, threadfin shad, and striped bass in autumn 2004 and analyzed samples representing the reservoir-wide, observed size range of each species. We dried tissue for 24–48 h at 60 °C, ground it to a powder, and shipped encapsulated samples to the University of California–Davis Stable Isotope Facility for a mass spectrometry determination of isotopic signatures. Ratios were evaluated and expressed as $\delta^{13}C$ and $\delta^{15}N$, per mil (‰) values relative to the ratios of the standards Pee Dee Belemnite and atmospheric nitrogen, respectively (Peterson and Fry 1987). Differences in stable isotope ratios between the species were tested using analysis of variance (ANOVA, $\alpha = 0.05$).

Shad size structure and predator gape limitation (H3, H4)

To test hypotheses H3 and H4, we used the fish sampling data described above to evaluate the growth rates and size structure of the gizzard shad population and assess the potential for gizzard shad to provide forage to striped bass and to be directly controlled by striped bass predation. We first estimated the maximum size of gizzard shad that striped bass were capable of consuming and then compared this with the observed size distribution of gizzard shad to assess gape limitation, defined here as the physical size restriction of a predator swallowing a prey. We collected scales, removed sagittal otoliths (from a subset representing the observed size range of each species), and measured the length and weight of all striped bass and gizzard shad caught in gill net sets. We estimated an average size-at-age for each age class of striped bass and gizzard shad in autumn of each year using scale-ageing techniques for striped bass (Secor et al. 1995) and a combination of otolith-ageing (Clayton and Maceina 1999) and analysis of length–frequency modes for gizzard shad.

Next, using these size-at-age data, we estimated the gape limitation of age-0 through age-6 striped bass on gizzard shad using the following equation (Jenkins and Morais 1978):

$$y_i = 0.000\,328x_i^2 + 0.128x_i + 27.9$$

where x equals the average total length (mm) of a striped bass of age i, and y equals the maximum total length (mm) of prey fish that a striped bass of age i would be able to consume. We then compared the estimated gape limit of striped bass with the size and age structure of both captured gizzard shad and threadfin shad to assess the potential for gizzard shad to become striped bass prey and to compare this relationship with an established predator–prey relationship. We also quantified an index of prey vulnerability (IOV) by dividing the number of gizzard shad less than the maximum prey size of each age class of striped bass by the total number of gizzard shad captured in autumn gillnetting and electrofishing, summarized as yearly averages.

Effects on the predator–prey cycle: bioenergetics modeling

Lastly, we used bioenergetics modeling to estimate potential changes in striped bass growth as a result of the recent gizzard shad introduction. We collected diet, thermal history, and growth data on striped bass and used that information to estimate consumption of shad using a fish bioenergetics model (Hanson et al. 1997). In this study, we used consumption estimates from Vatland (2006), coupled with hypothetical scenarios of potential gizzard shad effects, to predict potential growth responses in juvenile and adult striped bass (100–500 and >500 mm total length, respectively).

Our scenarios reflected two broad hypotheses of gizzard shad invasion and bracketed the range of possible outcomes: (*i*) gizzard shad enhanced the growth of striped bass, and (*ii*) gizzard shad were a detriment to striped bass growth (Table 1). These simulated scenarios were then compared with model outputs using parameters estimated for striped bass in Lake Powell from May to November 2004. Accordingly, we varied levels of the proportion of shad (*Dorosoma* spp.) in striped bass diets by season (spring, summer, autumn) as well as total consumption. The magnitude of these proportional changes was based on average annual differences in the proportion of shad in diets and total consumption estimates from 1985 to 2003 for juvenile and adult striped bass (Vatland 2006). In all three enhancement scenarios (E1–E3), we presumed that striped bass were able to consume most gizzard shad, assuming that striped bass were neither gape-limited nor spatially segregated from gizzard

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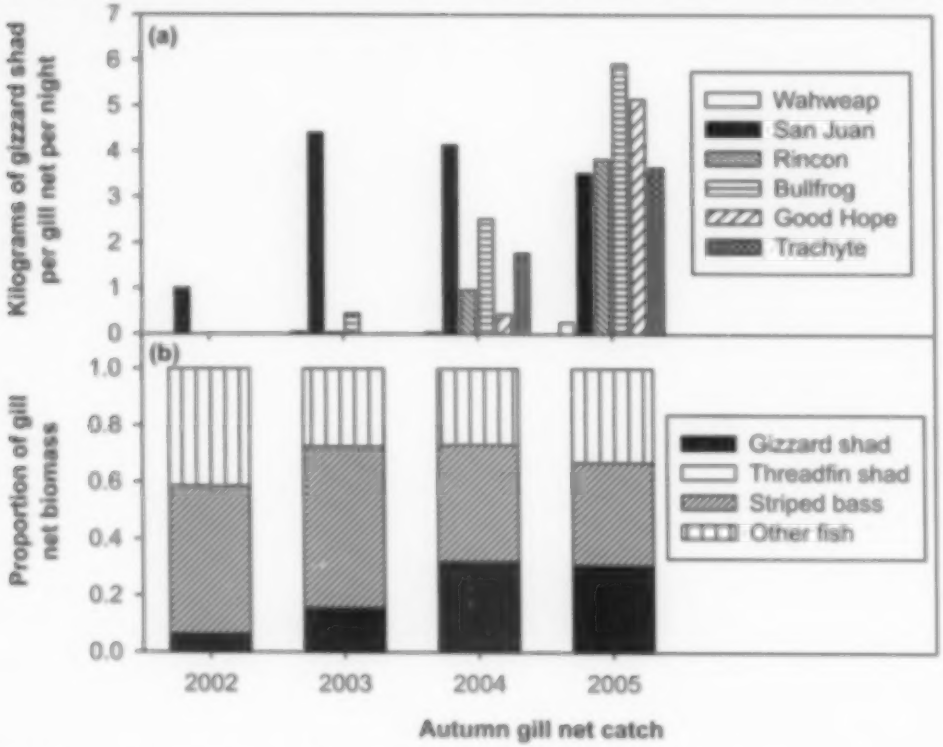
Fig. 3. (*a*) Biomass (kg) of gizzard shad (*Dorosoma cepedianum*) captured per standard gill net per night at six index sites and (*b*) proportion of gill net catch by taxanomic grouping from 2002–2005 autumn gill net surveys. Other fish include predominantly walleye (*Sander vitreus*), smallmouth bass (*Morone dolomieu*), channel catfish (*Ictalurus punctatus*), and common carp (*Cyprinus carpio*).



shad. In all of the detriment scenarios (D1–D4), we presumed that gizzard shad were not providing additional forage to striped bass in the summer and (or) autumn, and in some cases, gizzard shad were also presumed to negatively affect the availability of other prey, such as threadfin shad and zooplankton. To reflect the fact that gizzard shad often spawn earlier in the year than threadfin shad (Shelton et al. 1982) and may provide increased forage, we also simulated gizzard shad providing additional spring forage to striped bass in all the enhancement scenarios and in two detriment scenarios (E1–E3, D1–D2).

Results

Introduction and establishment stages

Gizzard shad distribution, biomass, and abundance (H1)

The distribution, biomass, and abundance of gizzard shad increased rapidly in Lake Powell from 2002 to 2005 (Fig. 3). In the autumn of 2002, 2 years after initial detection, gizzard shad distribution was limited to the upper sections of the San Juan Arm of Lake Powell (see Fig. 2), but by July 2003 their distribution extended to the mouth of the San Juan Arm. Gizzard shad were present throughout most of the reservoir by November 2003, and in 2004 and 2005, gizzard shad were captured during gill net surveys at all sample areas. This dispersal was unidirectional from the inflow of the San Juan Arm to its confluence with the historic Colorado River channel and then spread either up or down the reservoir, suggesting an isolated introduction location. In the San Juan Arm, CPUE (kilograms per net per night) of gizzard shad in autumn gill nets increased from 0.99 in 2002 to 4.4 in 2003 and decreased slightly to 4.1 in 2004 and 3.5 in 2005. Gizzard shad CPUE increased markedly in uplake sites after establishment throughout the reservoir in 2004 and widespread integration in 2005; the average gizzard shad CPUE for the Rincon, Bullfrog Bay, Good Hope Bay, and Trachyte Canyon areas (uplake sites) increased from 0.12 to 1.42 and 4.63 between autumn 2003, 2004, and 2005, respectively. Gizzard shad CPUE near the outflow area (Wahweap Bay) remained below 0.05 in 2003 and 2004 and below 0.25 in 2005. Within each sample area, relative abundance of gizzard shad caught in 2005 autumn gill nets was positively related to mean chlorophyll *a* values (spring, summer, and autumn) for littoral sites ($y = -2.11 + 2.06x$; $P = 0.037$, adjusted $R^2 = 0.74$) but not for pelagic sites ($P = 0.17$). Trends in the mean chlorophyll *a* values of both pelagic and littoral sites demonstrated a trophic gradient between riverine and lacustrine areas of the reservoir, with greater productivity in riverine areas.

Gizzard shad biomass relative to other fish species

The proportion of gizzard shad biomass, relative to other fish species caught in autumn gill nets, increased from 0.06 to 0.15 and 0.32, in 2002, 2003, and 2004, respectively, and leveled off at 0.31 in 2005 (Fig. 3). The proportional catch

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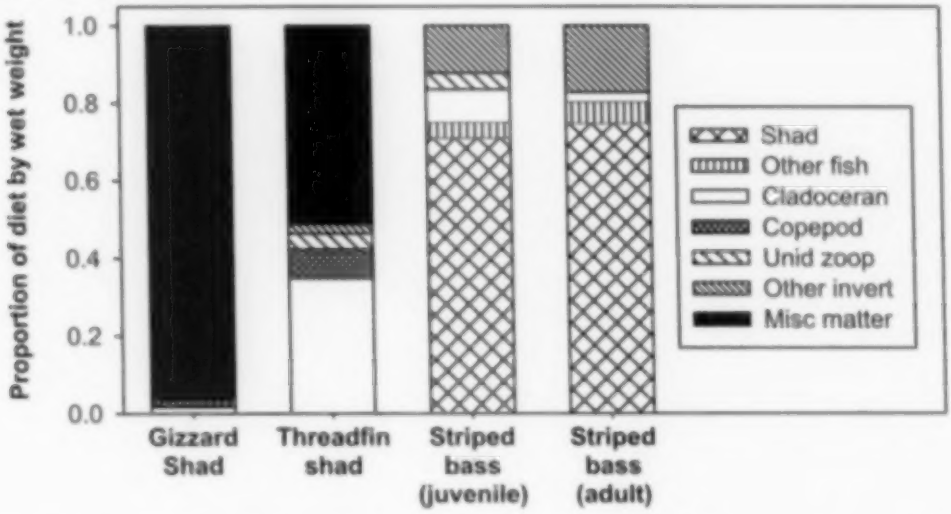
Fig. 4. Diet composition of fish stomachs collected in 2004, all sites and seasons combined. "Unid zoop" represents unidentified zooplankton; "Other invert" represents non-zooplankton invertebrates, such as chironomids, terrestrial insects, and crayfish; "Misc matter" includes phytoplankton, inorganic sediment, and organic sediment; "Shad" includes both gizzard shad (*Dorosoma cepedianum*) and threadfin shad (*Dorosoma petenense*).



of striped bass was similar in 2002 and 2003 at 0.52 and 0.57, respectively, but decreased to 0.41 and 0.36 in 2004 and 2005, respectively. The proportion of other fish species, predominantly walleye, smallmouth bass, channel catfish, and common carp, caught in autumn gill nets decreased from 0.41 to 0.28 in 2002 and 2003, respectively, and remained stable in 2004 at 0.27 but increased to 0.33 in 2005. The proportion of threadfin shad remained below 0.003 in each year. Biomass of catch (kg) for the four taxonomic groups (striped bass, gizzard shad, threadfin shad, and other fishes) was significantly different between years ($\chi^2 = 153$, df = 9, $P < 0.01$).

Integration stage

Zooplankton resource competition (H2)

The diet composition of gizzard shad, threadfin shad, and striped bass differed considerably in 2004 (Fig. 4). Gizzard shad consumed predominantly miscellaneous matter (algae, organic sediment, and inorganic sediment) and a small percentage of zooplankton (cladocerans, copepods, and unidentified zooplankton), whereas threadfin shad diets were composed of approximately half miscellaneous matter, half zooplankton (mostly cladocerans), and a small proportion of other invertebrates (mostly chironomids). Juvenile and adult striped bass diets were quite similar; both diets were composed mostly of shad (≥70%, primarily threadfin shad), followed by smaller proportions of other fish, other invertebrates, and zooplankton. Schoener's index of dietary overlap between gizzard shad and juvenile striped bass, threadfin shad and juvenile striped bass, and gizzard shad and threadfin shad were 3, 15, and 54, respectively, demonstrating no significant overlap in diets (Wallace 1981). Stable isotope ratios of ^{15}N were the highest in striped bass (164–569 mm total length) muscle tissue, indicating consumption at a higher trophic level, as compared with the two shad species (Fig. 5). Similarly, ratios of ^{15}N in threadfin shad (66–107 mm total length) muscle tissue were greater than that of gizzard shad (94–454 mm total length), indicating consumption at an intermediate trophic level for threadfin shad and consumption at a lower trophic level for gizzard shad (Fig. 5). Stable isotope ratios of ^{13}C were similar for striped bass and threadfin shad, with slightly higher ratios in gizzard shad muscle tissue, suggesting a possible difference in the ^{13}C enrichment of respective prey (Fig. 5). Differences in the ratio of ^{15}N between species were significantly different ($P < 0.001$), whereas the ratio of ^{13}C was not significantly different across fish species ($P = 0.29$).

Total zooplankton densities (including nauplii and rotifers, number·L^{-1}) in Lake Powell averaged 29 (3.8 standard error (SE)), 30 (5.6), and 50 (17) in the spring, summer, and autumn of 2004, respectively. The size distribution of zooplankton found in gizzard shad diets was generally composed of larger individuals than found in the environment, while the size of zooplankton found in threadfin shad diets more closely resembled the size distribution of the environment (Fig. 6). In 2004, the lengths of zooplankton found in gizzard shad diets, threadfin shad diets, and the environment averaged 1.1 (0.18–2.9, range), 0.76 (0.18–1.9), and 0.63 (0.12–7.8) mm, respectively, and differed significantly in pair-wise goodness-of-fit tests ($P < 0.01$).

Shad size structure (H3)

The overall size distribution of threadfin shad and gizzard shad differed greatly between species and gear type throughout this study (Table 2; Figs. 7 and 8). In particular, gizzard shad captured in gill nets were much larger than threadfin shad and attained sizes up to 496 mm in total length. The size distribution of fish captured by electrofishing was similar for both shad species, with gizzard shad being slightly larger but less abundant than threadfin shad. Finally, only threadfin shad (predominantly age-0) and no gizzard shad

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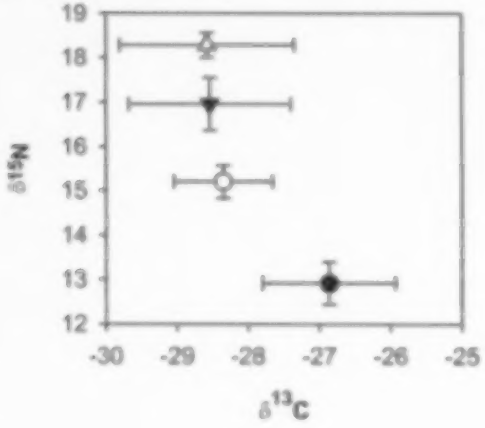
Fig. 5. Stable isotope ratios (^{15}N and ^{13}C, mean ± standard error (SE)) of gizzard shad (*Dorosoma cepedianum*; solid circle; n = 12), threadfin shad (*Dorosoma petenense*; open circle; n = 9), juvenile striped bass (*Morone saxatilis*; solid triangle; n = 5), and adult striped bass (open triangle; n = 6) collected in 2004 autumn gill nets. Juvenile striped bass were defined as being <500 mm in total length, with adult striped bass being ≥500 mm in total length.



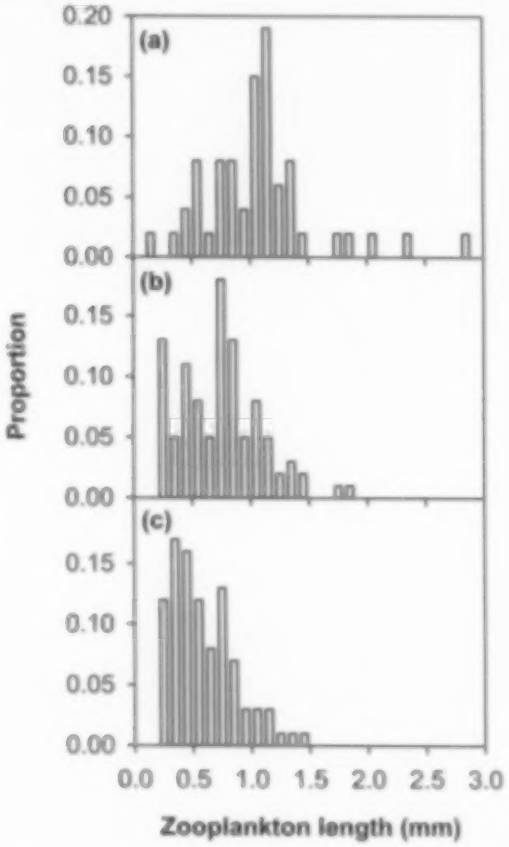
were captured in midwater trawling throughout 2002–2004. In 2005, a few gizzard shad were captured in midwater trawls, but the trawl catch was still predominantly composed of threadfin shad (96.6% by number by species).

The size distribution of threadfin shad varied little over the course of this study, whereas the size distribution of gizzard shad changed considerably as this invasion transitioned through the introduction, establishment, and integration stages (Fig. 7). Gizzard shad captured during the establishment stage in 2002 were relatively large adults, but this size range expanded in 2003 (in conjunction with an extensive dispersal) to include both larger and smaller fish. In 2004 and 2005, as gizzard shad completely dispersed and began integrating into the entire system, their overall size range remained relatively consistent. However, the proportion of smaller and younger gizzard shad increased markedly in 2004, and a cohort of gizzard shad around 350 mm in total length dominated the gill net catch in 2005. Age-0 gizzard shad were captured both by electrofishing in September and by gillnetting in October–November, and otolith-ageing indicated age-0 gizzard shad may obtain sizes as large as 191 mm in total length by November. Otolith-aged gizzard shad from 2003 and 2004 included five year classes (Table 3), but distinguishing year classes using length–frequency modes was ambiguous. Nonetheless, gizzard shad of all age classes exhibited relatively fast growth rates and a large maximum size in Lake Powell over the course of this study.

Predator gape limitations (H4)

Overall, striped bass predation on gizzard shad appeared to be gape-limited, especially for age-0 striped bass, whereas predation on threadfin shad was not gape-limited (Figs. 7 and 8). Based on estimated gape limits, age-1 and age-2 striped bass in 2003, 2004, and 2005 were capable of consuming age-0 gizzard shad, and age-3 and older striped bass

Fig. 6. Proportional length frequency of zooplankton found in (*a*) gizzard shad (*Dorosoma cepedianum*) and (*b*) threadfin shad (*Dorosoma petenense*) stomachs as compared with (*c*) zooplankton sampled from the reservoir in 2004; all sites and seasons combined.



were morphologically able to prey upon gizzard shad up to approximately 200 mm in total length. In contrast, most threadfin shad were within the gape limits of striped bass age-1 and older in all years of this study, and all ages of striped bass were physically capable of consuming threadfin shad captured in trawls. IOV estimates for gizzard shad as striped bass prey in the autumn of 2002, 2003, 2004, and 2005 were 0%, 17%, 46%, and 23%, respectively. The increase in IOV from 2002 to 2004 corresponded to the shift in gizzard shad population size structure to include a higher proportion of relatively small shad, whereas the decrease in IOV in 2005 corresponded to an increase in the proportion of larger gizzard shad, notably a strong cohort of shad approximately 350 mm in total length.

During the integration stage of this invasion (2004 and 2005), striped bass stomachs contained gizzard shad, threadfin shad, and unidentifiable shad with mean total lengths (mm) of 102 (63–178, range), 51 (35–92), and 55 (10–129), respectively (Fig. 9). The total length of these shad prey averaged 14% (5% standard deviation (SD)) of the total length of their striped bass predators. For striped bass greater than approximately 250 mm in total length, all shad prey re-

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Table 2. Mean catch per unit effort (CPUE, kilograms per net per night) and total length (mm) of shad (*Dorosoma* spp.) captured in July–August midwater trawls (TR), September electrofishing (EL), and October–November gillnetting (GN) in Lake Powell in 2002–2005.

		Mean CPUE (SE)*			Mean total length (SE)		
Species	Year	TR	EL	GN	TR	EL	GN
Threadfin shad	2002	16 (6)	44.6 (31.5)	0.10 (0.05)	44 (2.8)	111 (1.7)	145 (10.0)
	2003	1970 (587)	28.8 (8.7)	0.38 (0.23)	37 (2.4)	93 (2.5)	146 (1.8)
	2004	1721 (452)	72.2 (29.0)	0.93 (0.82)	35 (2.4)	104 (1.1)	103 (1.7)
	2005	2497 (1076)	6.5 (4.7)	0.62 (0.24)	34 (2.1)	74 (2.9)	140 (25.2)
Gizzard shad	2002	0 (—)	0 (—)	0.98 (0.98)	—	—	287 (2.0)
	2003	0 (—)	4.8 (4.8)	1.34 (1.02)	—	110 (2.6)	363 (7.4)
	2004	0 (—)	38.2 (29.4)	7.95 (2.87)	—	112 (3.9)	246 (5.7)
	2005	78 (40)	48.5 (26.6)	11.54 (1.12)	48 (5.7)	120 (3.6)	338 (6.9)

Note: Dashes (—) represent those values not available and (or) applicable.

*CPUE for trawling, electrofishing, and gillnetting was expressed as number of fish captured per trawl tow, electrofishing hour, and gill net night, respectively. SE, standard error.

mained within the estimated gape limit, but for striped bass less than approximately 250 mm in total length, a few unidentified shad prey were larger than the estimated gape limit.

Effects on the predator–prey cycle: bioenergetics modeling

We estimated a wide range of juvenile and adult striped bass growth rates across our bioenergetics scenarios (Fig. 10). The largest increases in striped bass seasonal growth, as compared with the 2004 baseline simulation, were observed in scenario E3 (increased striped bass forage in all seasons and increased total consumption), where juvenile and adult striped bass growth increased 75% and 314%, respectively. The largest decreases in estimated seasonal growth occurred under scenario D4 (decreased striped bass forage in the spring and autumn and decreased total consumption), where juvenile and adult growth decreased by 129% and 345%, respectively. Differences in the estimated growth of adults appeared to be closely and positively related to changes in total consumption. Juveniles responded to adjustments in total consumption as well, but were also noticeably affected by decreases in the proportion of shad in their summer and autumn diets. Neither adult nor juvenile simulations showed a strong growth response to the proportion of shad in their spring diets.

Discussion

Gizzard shad were first discovered in Lake Powell in 2000 near the inflow of the San Juan River, and by 2004 these fish had dispersed throughout the entire reservoir and were integrating into the aquatic food web. This rapid invasion provided a unique opportunity to track an introduced fish species through the introduction, establishment, and integration stages. By simultaneously examining aspects of the gizzard shad population, the other primary fish populations of Lake Powell, and the planktonic community, we were able to effectively integrate our present data with both long-term data on established fish populations in Lake Powell and an extensive body of literature on the ecology of gizzard shad. We used this integrated approach to evaluate the effects of gizzard shad on the aquatic food web and to assess generalities in the literature regarding the characteristics of successful and failed invasive fish species. We were also able to examine species-specific traits and assess their potential for predicting the effects of invasive fish species at different stages of the invasion process.

Gizzard shad introduction and establishment

The role of life-history characteristics

Gizzard shad became successfully established in Lake Powell and dispersed through the reservoir in just 4 years, demonstrating rapid dispersal, high growth rates, and large adult body size, all common characteristics of successful invaders (Moyle and Marchetti 2006). Propagule pressure (number of organisms introduced and number of introductions) or dose response is also considered critical to the successful introduction and establishment of deliberately and (or) incidentally introduced species (Ruiz and Carlton 2003; Marchetti et al. 2004*a*; Colautti et al. 2006). However, the first gizzard shad discovered in Lake Powell (2000) were presumably a small number of adults that migrated down the San Juan River to the reservoir following periodic flushes of the power plant at Morgan Lake, New Mexico (Mueller and Brooks 2004). Thus, based solely on propagule pressure, the establishment of gizzard shad in Lake Powell was unlikely; nonetheless, small numbers of adult gizzard shad, especially those of large body size, are capable of quickly establishing reproducing populations (Michaletz 1998*b*). As such, the successful establishment of gizzard shad in Lake Powell was likely influenced more so by their large body size and ability to reproduce rapidly than by the propagule pressure of their introduction. Our findings emphasize the important role life-history characteristics can play in the establishment of introduced fish species (e.g., Olden et al. 2006); here, large body size and high fecundity substituted for high propagule pressure (or dose; Ruiz and Carlton 2003) and resulted in a successful invasion of gizzard shad to the establishment stage.

The role of abiotic factors

Although the influence of abiotic factors is thought to be frequently underestimated in invasion ecology, as compared with biotic factors (Moyle and Light 1996*a*, 1996*b*), these physical characteristics can also affect the success of an invasive fish during the establishment stage (Marchetti et al.

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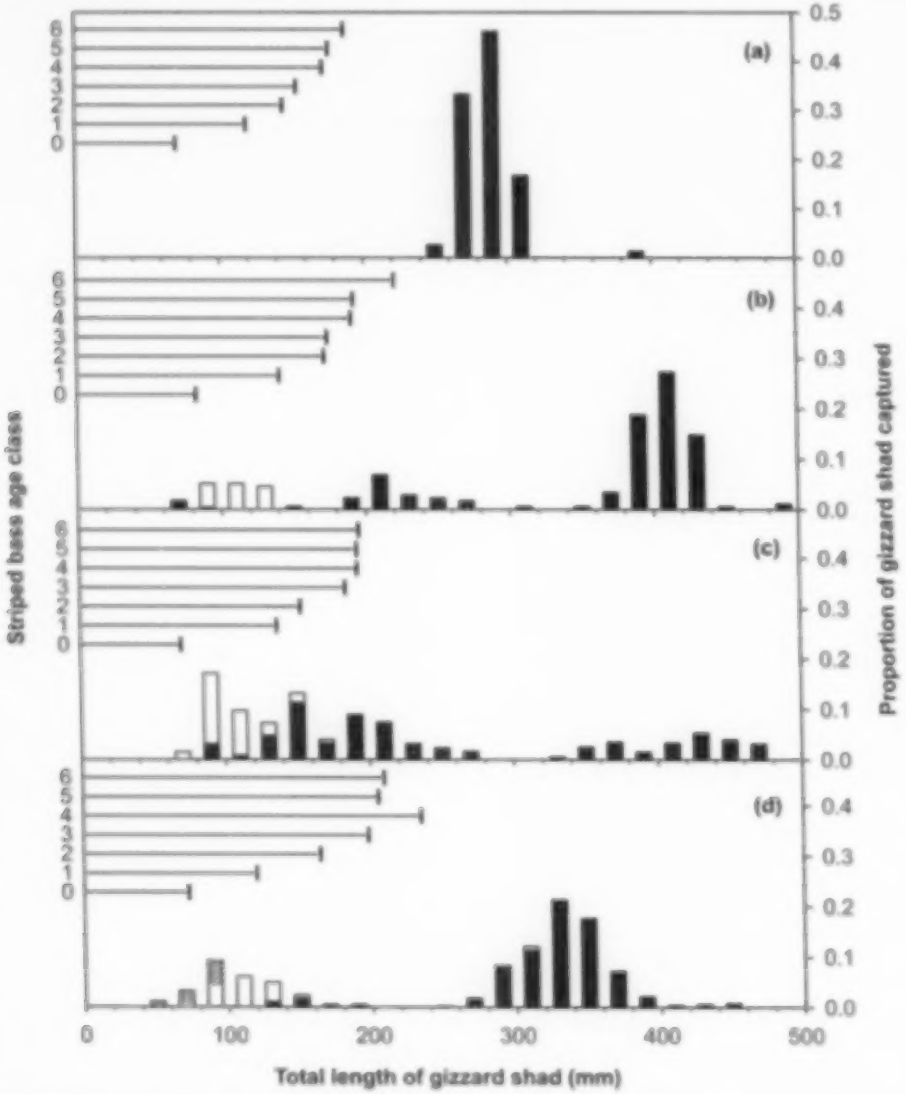
Fig. 7. Proportional length–frequency histograms of gizzard shad (*Dorosoma cepedianum*; 2002–2005, panels *a*–*d*, respectively) captured by trawling (hatched), electrofishing (open), and gillnetting (solid) in autumn fieldwork. Potential prey size range for striped bass (*Morone saxatilis*) is expressed by horizontal bars and based on year-specific mean size-at-age of autumn gill-net-captured striped bass and the gape calculations of Jenkins and Morais (1978).



2004*a*). Moyle and Light (1996*a*, 1996*b*) suggested the establishment of invasive salmonids in California was influenced by the ability of these fish to adapt to the local hydrologic regime. Similarly, *Dorosoma* spp. are sensitive to the thermal regime of their environment, and this vulnerability to cold water temperatures commonly causes winter kills in the northern range of both gizzard shad and threadfin shad (Griffith 1978). Stein et al. (1995) further suggested that winter mortality of established gizzard shad populations limits their dominance in some north-temperate lakes and reservoirs. Winter kills of gizzard shad in Lake Powell are highly unlikely, given the depth (>30 m); relatively warm, winter water temperatures of this southwestern United States reservoir (9.0 °C average annual minimum surface temperature); and the generally suitable dissolved oxygen concentrations. Further, a high level of human disturbance, such as occurs in Lake Powell, provides a physical environment in which invasive species are frequently successful (Hobbs 1989; Havel et al. 2005; Olden et al. 2006). The types of physical factors found in Lake Powell (e.g., warm climate, high human impact) are common to other large regional areas that have experienced a large number of successful fish invasions, including, for example, the states of California and Florida (Fuller 2003; Marchetti et al. 2004*b*). As demonstrated here, consideration of physical aspects of the receiving system provide reasonably accurate predictions of invasion success (Fuller 2003; Ruiz and Carlton 2003), especially when these abiotic factors are considered in combination with life-

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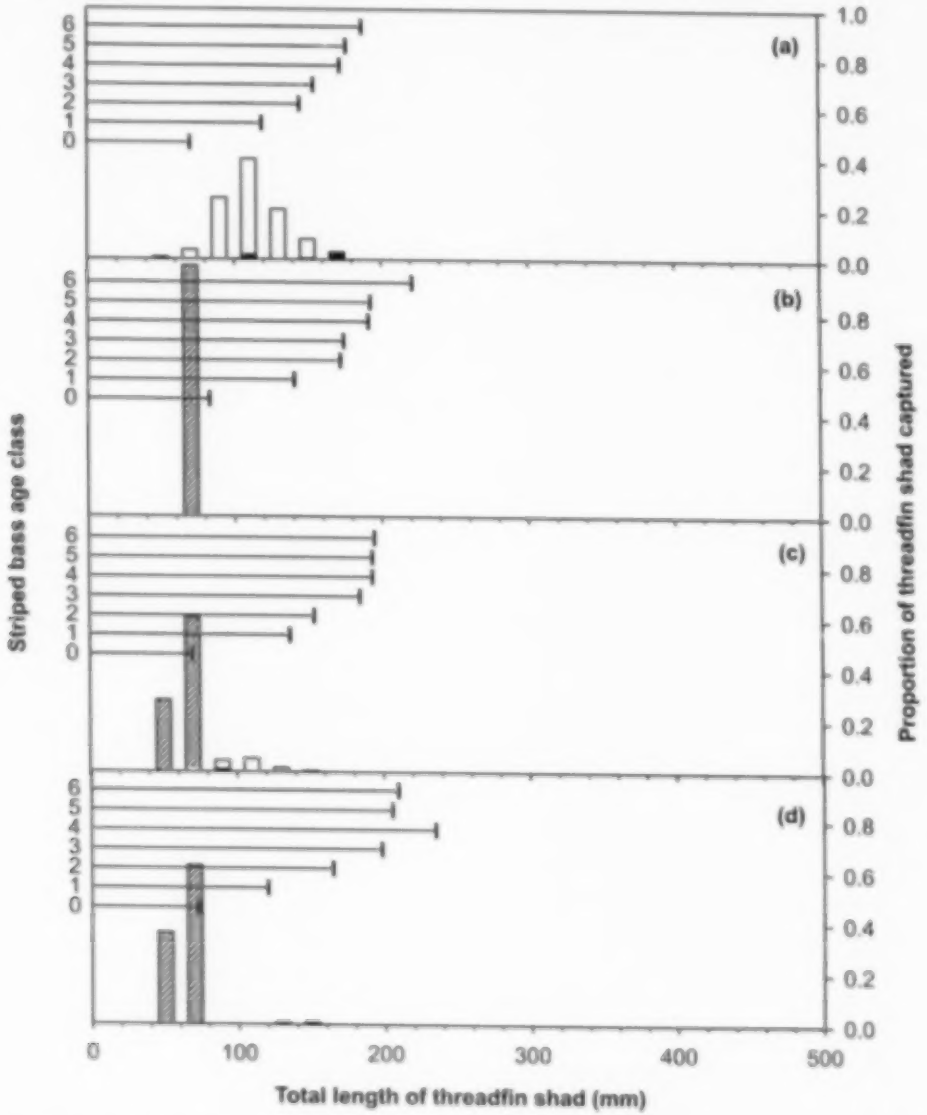
Fig. 8. Proportional length–frequency histograms of threadfin shad (*Dorosoma petenense*; 2002–2005, panels *a*–*d*, respectively) captured by trawling (hatched), electrofishing (open), and gillnetting (solid) in autumn fieldwork. Potential prey size range for striped bass (*Morone saxatilis*) is expressed by horizontal bars and based on year-specific mean size-at-age of autumn gill-net-captured striped bass and the gape calculations of Jenkins and Morais (1978).



history traits. After introduction, this combination of both life-history traits, such as high fecundity and large body size, and a lack of physical environmental constraints within the reservoir further contributed to the rapid dispersal and establishment of gizzard shad in Lake Powell.

The role of predatory control

Following introduction and establishment in the San Juan Arm, gizzard shad spread swiftly through the reservoir and increased their relative abundance and biomass considerably over only 4 years. While life-history traits and physiological tolerance undoubtedly control the establishment of invasive fish species (Marchetti et al. 2004*a*), additional factors clearly influenced the widespread establishment of gizzard shad in Lake Powell. Most importantly, the initial population established in the San Juan Arm of the reservoir was composed of mostly large adults >200 mm in total length, much larger than the gape limitation of most striped bass predators in the reservoir. Thus, the dispersal of adult gizzard shad within Lake Powell was not initially limited by predation (i.e., enemy release hypothesis; but see Colautti et al. 2004), a factor that can pose a critical constraint to introduced fish species, especially in systems with dominant piscivores or smaller, more vulnerable prey (e.g., Szendrey and Wahl 1996). Accordingly, the rapid dispersal of gizzard shad in Lake Powell would likely have been slowed if the

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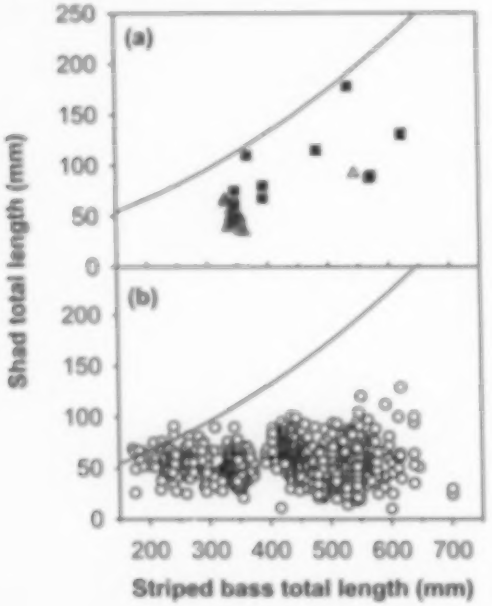
Table 3. Mean length-at-age of gizzard shad (*Dorosoma cepedianum*) from 2003 and 2004 autumn gillnetting.

	Gizzard shad total length (mm)		
Age	Mean	SE	*n*
0	171	6.9	5
1	246	11.5	7
2	386	18.7	3
3	411	6.0	11
4	446	18.3	4

Note: Age was determined by otolith ageing; *n* equals the number of shad that were aged. SE, standard error.

Fig. 9. Striped bass (*Morone saxatilis*) total length (mm) vs. total length of shad (*Dorosoma* spp.) prey found in the stomachs of striped bass captured during gill net surveys in 2004 and 2005 (all seasons combined). Shad prey are identified as (*a*) threadfin (*D. petenense*; open triangle), gizzard (*D. cepedianum*; solid square), or (*b*) unidentified shad (open circle). The lines represent the estimated gape limitation of striped bass based on Jenkins and Morais (1978).

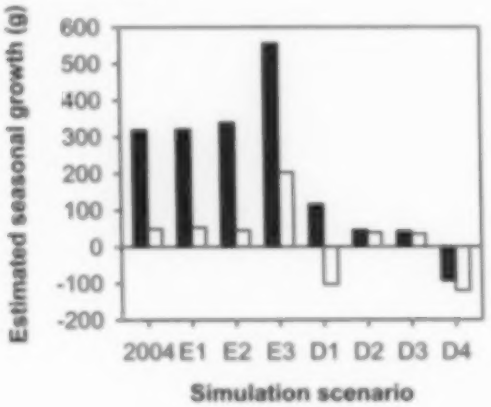


initial population of gizzard shad had been composed of smaller individuals and (or) individuals that grew more slowly (i.e., smaller individuals that would have been more susceptible to predatory control). These results point to the importance of species interactions (i.e., with enemies; Colautti et al. 2004), especially predatory control, in determining the success of an invader at the establishment stage (Moyle and Light 1996*a*, 1996*b*).

The role of primary productivity (H1)

As this invasion transitioned through the establishment stage, our ability to predict both the success and effects of invasion became more challenging; however, exploration of potential mechanisms operating at different trophic levels

Fig. 10. Bioenergetics-based estimates of adult (solid bars) and juvenile (open bars) striped bass (*Morone saxatilis*) seasonal growth (May–November) in 2004 and under various enhancement (E) and detriment (D) scenarios. Scenarios were based on potential effects of gizzard shad (*Dorosoma cepedianum*) introduction in Lake Powell.



did enhance our understanding of the process. In accordance with our initial predictions, the greatest increases in relative abundance and biomass of gizzard shad were observed in the most productive areas of the reservoir, even though some of those productive areas were substantial distances (over 160 river km) from the initial point of invasion. This pattern was consistent with previous research, as a positive relationship between gizzard shad density (or biomass) and primary productivity has been observed in a variety of freshwater systems in the USA, including natural lakes (Allen et al. 2000) and reservoirs (Bayne et al. 1994; Michaletz 1998*a*; Bremigan and Stein 1999). Buynak et al. (2001) also observed this trend during artificial fertilization experiments in a large warm-water reservoir. We suspect this strong relationship results because gizzard shad are primarily consuming algal matter or derivatives thereof, and we would not expect to see such a tight relationship for a fish species that feeds at a higher trophic level (Carpenter and Kitchell 1993). We suggest that careful attention be paid to the trophic gradient found in several reservoirs, as this gradient can greatly influence the distribution of food resources for invasive fishes and thus their pattern of establishment and integration (Hall and Van Den Avyle 1986; Michaletz and Gale 1999; Okada et al. 2005). Thus, an index of primary productivity (chlorophyll *a*), which is both simple to collect and analyze and closely tracks the trophic gradient of a reservoir, provided a useful and predictive tool in assessing the invasion extent and distribution of this invasive and omnivorous fish species.

Gizzard shad integration

Zooplankton resource competition (H2)

As gizzard shad continued to integrate into the Lake Powell food web, we also considered the potential for competition among gizzard shad, threadfin shad, and juvenile striped bass over zooplankton resources. Overall, average zooplankton densities in Lake Powell are low relative to

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other more productive systems (i.e., zooplankton density increases with productivity; Bays and Crisman 1983; Pinto-Coelho et al. 2005), such that zooplanktivores in Lake Powell may be food-limited (e.g., Michaletz 1999). However, as discussed above, Lake Powell is an extremely heterogeneous (and large) system with varying levels of productivity, and the overall zooplankton densities reported here may underestimate true food availability for zooplanktivores, especially in littoral areas. In addition, relative to other systems (Carlander 1969, 1977; Linam and Kleinsasser 1987), threadfin shad demonstrated high measures of condition in Lake Powell during the course of this study (Budy et al. 2005), despite relatively high densities. While these observations would suggest that zooplankton were not limiting for threadfin shad, if gizzard shad and (or) striped bass were predominantly zooplanktivorous, all three fish species could be competing for food during some life stages, and the increased forage on secondary production could limit the availability of zooplankton as prey.

Contrary to our original predictions, however, based on diet data; the size distribution of consumed zooplankton; and isotopic properties for gizzard shad, threadfin shad, and juvenile striped bass, the potential for competition over zooplankton resources appears minimal. Gizzard shad are capable of diminishing zooplankton resources and then successfully switching to alternative algal and detrital food sources (DeVries and Stein 1992); intense feeding on zooplankton is most likely to occur with age-0 gizzard shad 30–60 mm in total length (Yako et al. 1996; Dettmers et al. 1998). In 2004, the size distribution of zooplankton consumed by gizzard shad in Lake Powell was composed of larger individuals than those sampled from the environment and those consumed by its cogener, threadfin shad; as larger zooplankton consumed by gizzard shad are energetically richer than the smaller zooplankton consumed by threadfin shad (Snow 1972), gizzard shad could have a competitive edge over threadfin shad. However, these gizzard shad, mostly large adults, were primarily consuming algal and detrital matter (zooplankton make up only a small portion of their diet), and consequently, their overall impact on the zooplankton population is limited. Similarly, both adult and juvenile striped bass rarely consumed zooplankton, although we note this trend could change in years with limited shad forage (i.e., striped bass may switch from piscivory to invertivory; Gustaveson 1999; Nelson et al. 2003).

Our conclusion that competition among these three species will be limited through the integration stage is supported not only by our diet evaluation, but also by the clear difference in stable isotope ratios of ^{15}N. These data demonstrate that gizzard shad feed opportunistically at lower trophic levels; the omnivorous nature of gizzard shad is a biological characteristic shared by many successful invaders (e.g., common carp; Moyle and Marchetti 2006). It is very important to note, however, that our data are not conducive to evaluating the potential for competition at larval or juvenile life stages of both shad species, such that it remains possible that competition could be occurring at these early life stages (e.g., Bremigan and Stein 2001). Nonetheless, our observations of minimal potential for competition parallel the observations of Davis (2003), who suggested that intertrophic interactions that result from invasions (e.g., predation) pose a much greater threat to resident species than intratrophic interactions, such as competition.

In addition to differences in feeding preference and thus trophic position, it appeared that gizzard shad and threadfin shad were also spatially segregated during the course of this study. Age-0 threadfin shad were abundant in 2003, 2004, and 2005 summer trawls, but no gizzard shad were captured in these pelagic samples in 2003 and 2004 and only a limited number in 2005. Gizzard shad were abundant in the more littoral gill net catches, and there was a slightly greater abundance of gizzard shad than threadfin shad caught in nearshore electrofishing samples. Stable isotope ratios of ^{13}C also suggested a difference in prey sources for the two shad species, with higher ratios of ^{13}C in gizzard shad muscle tissue than in threadfin shad and striped bass tissue, a dissimilarity that could be attributed to differences in littoral versus pelagic prey (e.g., France 1995). Accordingly, Baker and Schmitz (1971) suggested threadfin shad are more limnetic than gizzard shad when feeding. The combination of these factors strongly suggests spatial segregation occurs between gizzard shad and threadfin shad populations in Lake Powell, with gizzard shad tending toward littoral habitat and threadfin shad toward pelagic habitat. Although, spatial segregation among striped bass and both shad was not explicitly considered in this part of the study (striped bass were only targeted using gill nets), competition over zooplankton resources appeared to be limited as a result of spatial segregation and (or) diet preferences. Thus, the combination of the ability to feed opportunistically (e.g., omnivory) and the large size and heterogeneous nature of this ecosystem (e.g., spatial segregation) made our predictions of invasion effects during the integration stage more tenuous.

Shad size structure (H3)

In contrast with the predictable and consistent relationship between primary productivity and gizzard shad density and biomass in Lake Powell, the relationship between primary productivity and gizzard shad growth was more complex. Generally, age-0 gizzard shad growth increases with primary productivity, a positive relationship attributed to increased food availability (Michaletz 1999; Bremigan and Stein 2001). However, age-1 and older gizzard shad often demonstrate the opposite pattern, where growth decreases with increasing primary productivity, a pattern attributed to density-dependent mechanisms such as resource competition (DiCenzo et al. 1996; Michaletz 1998*a*; Clayton and Maceina 2002). As such, we expected adults to grow quickly and juveniles slowly in an oligotrophic system such as Lake Powell, but both adult and juvenile gizzard shad grew rapidly over the course of this study. We did observe a shift in the overall size distribution toward smaller gizzard shad in 2003 and 2004; however, age-0 gizzard shad still attained sizes up to 191 mm in total length by November. The increase in younger, smaller shad and their relatively fast growth rates appeared to be due to the initial effects of dispersal and reproduction in new areas of the reservoir (i.e., limited intraspecific competition). In 2005, by which time gizzard shad were well-established and integrating into Lake Powell's aquatic ecosystem, this introduced fish population was dominated by large fish, corresponding with our initial predictions. We note also that although Lake Powell is generally characterized as oligotrophic, it is a very

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large, heterogeneous system with strong trophic gradients of high to low productivity, not only across the reservoir but also from littoral and pelagic zones (Mueller and Horn 1999; this study). This heterogeneity provides opportunities for fish to choose from a wide variety of habitat types and thus limits the utility of static categorizations of trophic status in predicting some aspects of invasion success (e.g., here, fish growth rates during establishment and then integration).

Further, Lake Powell, like many lentic systems, is dynamic with several factors, in addition to primary productivity, that could influence the gizzard shad population size structure over time. Abiotic conditions, such as changes in water elevation and temperature, can directly affect larval shad recruitment (Michaletz 1997) and, subsequently, contribute to shifts in population size structure. As gizzard shad continue to integrate into Lake Powell's aquatic community, density-dependent factors could slow their growth; however, most observations of density-dependent reductions in growth of gizzard shad have occurred in systems that are more productive than Lake Powell (DiCenzo et al. 1996; Michaletz 1998*a*; Clayton and Maceina 2002). Accordingly, we predict that this invasive species will continue to increase in density and biomass, especially in the most productive inflow areas of Lake Powell, but these increases will level off in a few years. This prediction corresponds with initial observations of gizzard shad in the San Juan Arm of the reservoir, where autumn catches of this established population remained consistent between 2003 and 2005, and growth rates remained relatively high. Thus, it appears that during the integration stage, juvenile gizzard shad will continue to grow rapidly, at least in the absence of complex recruitment dynamics (not considered here), which clearly warrant further research (e.g., Peterson et al. 2004).

Predator gape limitations (H4)

Consideration of the growth rate of an invader can also be critical to understanding their potential as forage, as prey growth may determine whether or not an invading animal will become forage or will be free from predation at higher trophic levels. Over time, the proportion of gizzard shad potentially vulnerable to striped bass predation increased as the size distribution of gizzard shad shifted to include more age-0 gizzard, but only a small proportion of gizzard shad was potentially available as forage for age-0 striped bass. Age-1 and older striped bass were not gape-limited in consuming age-0 gizzard shad in 2004, but few striped bass consumed shad prey longer than approximately 120 mm in total length, well below the estimated gape limit of larger striped bass. Juanes (1994) suggested that piscivores frequently consume prey much smaller than their maximum gape limitation, and Overton (2003) similarly described the optimum prey size for striped bass as 21% of the predator's total length. During the course of this study, the total length of shad consumed by striped bass in Lake Powell averaged 15% of the predator's total length. Correspondingly, analyses of striped bass diets in several systems have supported a preference for prey considerably smaller than their maximum gape limit (Bonds 2000; Overton 2003; Walter and Austin 2003), a pattern supported by our predator–prey size data; we observed a concentration on smaller forage, in this case age-0 shad. The extent to which gizzard shad provided additional forage for striped bass in Lake Powell depended largely on the size and thus growth of the age-0 gizzard shad cohort. Our ability to directly compare relative densities of gizzard shad and threadfin shad was somewhat limited, because gear types were disproportionately successful at capturing the two species. In addition, other factors, such as spatial segregation, could limit the availability of gizzard shad as forage for striped bass (e.g., thermal preference). Nonetheless, there appeared to be much higher densities of age-0 threadfin shad available to striped bass predators. In this case, age-0 gizzard shad appear to be limited in numbers, and those that were present quickly outgrew predator gape limits, providing limited forage to the striped bass population, a critical factor influencing the integration stage of this invasion as well as many invasions elsewhere (Ruiz and Carlton 2003; Colautti et al. 2004).

Bioenergetic predictions and the predator–prey cycle

Finally, as gizzard shad continued to integrate into Lake Powell's aquatic ecosystem, bioenergetics-based modeling provided us with an additional means of assessing the potential future impacts of this introduced species on the economically valued striped bass population. Through our predictive model scenarios, we were able to gain a better understanding of the potential magnitude of growth response expected from striped bass, as a result of gizzard shad introduction. The potential growth responses were considerable, especially given that the narrow range of parameter adjustments used for the different scenarios was based on empirical preinvasion data. These simulations suggest that juvenile striped bass are most sensitive to decreases in the availability of shad in the summer and autumn. This, in combination with gape limitation in juvenile striped bass when preying on gizzard shad in Lake Powell, suggests juvenile striped bass are more likely to be effected negatively by gizzard shad than adult striped bass. However, the potentially negative effects on juvenile striped bass could still be consequential to other trophic dynamics, including the valued fishery of Lake Powell (e.g., direct and indirect effects; Ives et al. 1999), as these juveniles have a disproportionate influence on striped bass population dynamics and the predator–prey cycle of Lake Powell's pelagic food web (Vatland 2006).

Over the past 30 years, Lake Powell's striped bass and threadfin shad populations have fluctuated dramatically in abundance, and the dynamics of these two populations appear to be closely related (Vatland 2006). McCann et al. (2005) suggest that when mobile, higher-order consumers, such as striped bass, are tightly coupled with local habitats, these predators can have a destabilizing effect on food webs. During some time periods in Lake Powell, an imbalance between the predatory demand of the striped bass population and the forage supply of the threadfin shad population has resulted in large boom–bust cycles in the striped bass fishery (Vatland 2006). Hypothetically, the (incidental) introduction of an additional forage fish, such as gizzard shad, could provide stability to a predator–prey cycle (Gido et al. 2000), if the invader provided substantial forage. This additional forage may then act to release the primary prey, in this case threadfin shad, from the intense predation pressure assumed

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to, in part, drive the dramatic predator–prey cycle observed in this system (Vatland 2006). In addition, the apparent tendency of gizzard shad to feed in more littoral and benthic habitat suggests they may act as important integrators of benthic energy to the pelagic food web (e.g., Vander Zanden and Vadeboncoeur 2002). However, the results of this study suggest that gizzard shad are providing only limited forage for striped bass, especially juvenile striped bass, which have a large consumptive demand on the threadfin shad forage base. As such, gizzard shad could actually intensify this predator–prey cycle by competing with both threadfin shad and juvenile striped bass in years of low, overall productivity. Further, the unpredictable nature of reservoir environments and the potentially mismatched evolutionary history of introduced species assemblages (e.g., Gido et al. 2000) limited our ability to predict the long-term effects of gizzard shad integration into the Lake Powell food web.

In summary, gizzard shad became successfully established in Lake Powell and spread throughout this large, heterogeneous reservoir within 4 years. This relatively quick establishment and extensive dispersal was clearly aided by an ability to grow quickly, exceed gape limits, and reproduce rapidly, combined with a lack of environmental constraints. As gizzard shad dispersed throughout the reservoir and began to integrate into the aquatic food web, the highest densities of this invader were observed in the more productive riverine areas and corresponded positively with littoral chlorophyll *a* concentrations; this relationship provided us with a useful and predictive tool for assessing invasion extent. Fast growth rates, consequent predator gape limits, and limited potential for competition combined and proved highly influential in determining invasion success. We predict gizzard shad will continue to be successful invaders of Lake Powell as they fully integrate into the aquatic ecosystem; however, their introduction will have only limited effects on the established striped bass – threadfin shad predator–prey cycle (assuming the abiotic conditions of this system do not fluctuate dramatically). By examining a suite of biological characteristics and the physical environment, we demonstrate that the effects of an invader may be predictable through the stages of introduction and establishment; however, at the integration stage of invasion, predicting invasion effects can be more challenging, especially for omnivorous species and in large heterogeneous systems that offer opportunities for spatial segregation.

Acknowledgements

This work was funded by the Utah Department of Natural Resources – Division of Wildlife Resources (Project XII, Sport Fisheries Research, Grant No. F-47-R, Segment 16), the US Geological Survey – Utah Cooperative Fish and Wildlife Unit, Vice President for Research at Utah State University (fellowship for Shane Vatland). Numerous technicians assisted with field work, and Susan Durham and Patty Kuhns provide statistical and technical support. Gary Thiede provided extensive logistical, field, and laboratory oversight, as well as assisting with manuscript development. Peter McHugh, Wayne A. Wurtsbaugh, James W. Haefner, and George Blommer all reviewed previous drafts of this manuscript.

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Multiscale effects of flow regime and habitat and their interaction on fish assemblage structure in eastern Australia

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Abstract: We examine the multiscale influence of environmental and hydrological features of the riverine landscape on spatial and temporal variation in fish assemblages in eastern Australia. Multiresponse artificial neural network models provided accurate predictions of fish assemblages in the Mary River based on species presence–absence data (mean Bray–Curtis similarity between predicted and observed composition = 84%) but were less accurate when based on species relative abundance or biomass (mean similarity = 62% and 59%, respectively). Landscape- and local-scale habitat variables (e.g., catchment area and riparian canopy cover) and characteristics of the long-term flow regime (e.g., variability and predictability of flows) were more important predictors of fish assemblages than variables describing the short-term history of hydrological events. The relative importance of these variables was broadly similar for predicting species occurrence, relative abundance, or biomass. The transferability of the Mary River predictive models to the nearby Albert River was high for species presence–absence (i.e., closer match between predicted and observed data) compared with species abundances or biomass. This suggests that the same landscape-scale features are important determinants of distribution of individual species in both rivers but that interactions between landscape, hydrology, and local habitat features that collectively determine abundance and biomass may differ.

Résumé : Nous examinons à des échelles multiples l'influence des caractéristiques environnementales et hydrologiques d'un paysage de rivière sur la variation spatiale et temporelle des peuplements de poissons en Australie orientale. Des modèles de réseau neural artificiel à réponses multiples génèrent des prédictions précises des peuplements de poissons dans la rivière Mary à partir des données de présence et d'absence des espèces (similarité moyenne de Bray–Curtis entre les compositions prédites et observées = 84 %), mais elles sont moins exactes lorsqu'elles se basent sur l'abondance relative ou la biomasse des espèces (similarités moyennes respectives de 62 % et de 59 %). Les variables à l'échelle du paysage ou à l'échelle locale (par ex., la surface du bassin versant et la couverture de la canopée de la rive) et les caractéristiques du régime d'écoulement à long terme (par ex., la variabilité et la prédictibilité des débits) sont de meilleures variables explicatives des peuplements de poissons que les variables qui décrivent le déroulement à court terme d'événements hydrologiques. L'importance relative de ces variables est grosso modo semblable pour la prédiction de la présence, de l'abondance relative et de la biomasse des espèces. Les modèles prédictifs mis au point sur la rivière Mary peuvent être utilisés sur la rivière Albert avoisinante avec plus de succès (c'est-à-dire avec un meilleur accord entre les valeurs prédites et observées) dans le cas de la présence–absence des espèces que dans celui des abondances et des biomasses des espèces. Ces observations indiquent que les mêmes facteurs à l'échelle du paysage sont importants dans la détermination de la répartition des espèces individuelles dans les deux rivières, mais que les interactions entre le paysage, l'hydrologie et les caractéristiques locales de l'habitat qui déterminent ensemble l'abondance et la biomasse peuvent être différentes.

[Traduit par la Rédaction]

Introduction

Understanding the mechanisms that structure riverine communities requires a multiscale approach that recognizes the nested physical hierarchy of natural river systems (Fausch et al. 2002; Lowe et al. 2006). Ecological patterns observed at small scales are the product of both local habitat structure and dynamics and constraints imposed at broader spatial and tem-

Received 29 September 2006. Accepted 4 June 2007. Published on the NRC Research Press Web site at cjfas.nrc.ca on 29 September 2007.
J19564

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 doi:10.1139/F07-108 © 2007 NRC Canada

poral scales. In this context, riverine flow regimes are a key driver of lotic ecosystem structure and function (Poff et al. 1997; Bunn and Arthington 2002) and their hierarchical influence may be manifested at multiple spatial and temporal scales (Biggs et al. 2005). Long-term variations in flow magnitude, and the timing, frequency, and duration of flow events, define the physical habitat template over large spatial scales (e.g., catchments and subcatchments), whereas the short-term history of hydrological events influences habitat availability and connectivity at smaller spatial scales (e.g., within and among river reaches). These factors collectively influence the availability of refuges, food resources, opportunities for movement and migration, and conditions suitable for spawning and recruitment of fish and other biota (e.g., Schlosser 1991; Humphries et al. 1999; Magoulick and Kobza 2003).

To date, many of the hierarchical linkages in riverine ecosystems are poorly understood and inadequately validated and remain expressed largely as descriptive hypotheses rather than as predictive or quantitative models. In this paper, we use a hierarchical conceptual framework to address two issues that have impeded efforts to link variation in local fish assemblages to the independent and interactive effects of hydrological variation with the physical features of stream channels at multiple scales. First, the utility of multiscaled approaches is increasingly recognised, yet few empirical studies have explicitly attempted to characterise environmental processes influencing local assemblages simultaneously through both space and time (Lowe et al. 2006). Typically, species–environment relationships are evaluated at one or more spatial or temporal scales, but not both (Jackson et al. 2001). Second, the manner in which biological assemblages are characterised may influence the strength and nature of species–environment relationships detected (Cushman and McGarigal 2004); however, this has received little attention in studies of aquatic ecosystems. For example, species occurrence at a particular location and time may be due to a combination of large-scale factors (e.g., climate and catchment morphology), whereas the relative abundance or biomass of species in an assemblage is more likely driven by processes operating at the local scale (Rahel 1990).

We conducted our study in a region of eastern Australia characterised by highly variable and unpredictable flow regimes and, hence, challenging conditions for predictive model development (Pusey et al. 2000; Kennard et al. 2006*a*). Spatial and temporal patterns of fish assemblage structure in the Mary River, southeastern Queensland, were expressed as three biotic measures (species presence–absence, species relative abundance, and species relative biomass). These measures were modelled as a function of a hierarchy of environmental and hydrological variables using multiresponse artificial neural networks (Olden 2003). We then tested the transferability of the Mary River predictive models on a smaller set of independent data from the nearby Albert River. Our ultimate objectives were to gain insight into the relative influence of multiscaled environmental and hydrological features of the riverine landscape on different properties of fish assemblages and to evaluate the generality of these relationships among nearby catchments.

Materials and methods

Study area

Southeastern Queensland, Australia, is a region of transitional temperate to subtropical climate constituting a single freshwater fish biogeographic region (Unmack 2001). The majority of rainfall and streamflow occur in the summer months of January to March, often followed by a second minor discharge peak between April and June. Inter- and intra-annual variation in discharge is substantial, where high and low flows may occur at any time of year (Pusey et al. 2004). Our study systems, the Mary River (9400 km^2) and Logan–Albert River (4195 km^2) catchments, drain to the eastern coast of Australia and are bounded to the west by extensions of the Great Dividing Range (Fig. 1). Sampling sites were arrayed widely throughout each catchment (located on third- to seventh-order streams and rivers) to encompass as much of the natural biological and environmental variation as possible. Sites were selected according to a stratified random sampling design (i.e., randomly stratified by relative position within the catchment), which was constrained by available access points to the river, water depth (see below), and a desire to avoid areas affected by human activity. Reaches chosen for study represented the best condition available (sensu Hughes 1995) (i.e., minimally disturbed riparian vegetation and bank and channel structure in natural condition), and the degree of flow regulation was minimal. Human impacts were therefore not expected to influence fish assemblage dynamics and relationships with flow and habitat described in our study. Sixteen sites in the Mary River were sampled seasonally (Austral winter, spring, and summer) on nine or 10 occasions between 1994 and 1997 (n = 158 samples). Eleven sites in the Albert River were sampled seasonally on six or seven occasions between 1995 and 1997 (n = 71 samples).

Fish sampling

Fish surveys were conducted in wadeable stream reaches (<1.5 m maximum depth). We avoided sampling immediately after high flow events, and on occasions when high flows occurred, we waited at least 2 weeks until flows had subsided and fish sampling could be conducted efficiently. Study sites were between 70 m and 80 m of stream length, usually constituting an entire meander wavelength or one riffle–run–pool sequence. We sampled three contiguous individual mesohabitat units (i.e., riffles, runs, or pools) within each reach and combined this data to represent each site. Each mesohabitat unit was blocked upstream and downstream with weighted seine nets (11 mm stretched-mesh) to prevent fish movement into or out of the study area. Fish assemblages were intensively sampled using multiple-pass electrofishing (Smith-Root model 12B Backpack Electrofisher; Smith-Root Inc., Vancouver, Wash.) and seine netting (11 mm stretched-mesh) (see Kennard et al. (2006*b*) for a complete description and evaluation of the sampling protocol). All fish collected were identified to species, counted, and measured (standard length to the nearest millimetre). Australian native fish were released at the point of capture, whereas nonindigenous species were sacrificed (using benzocaine) in accordance with the Queensland Fisheries Act (Queensland Parliamentary Council 1994). The weight of each fish was estimated by reference to published rela-

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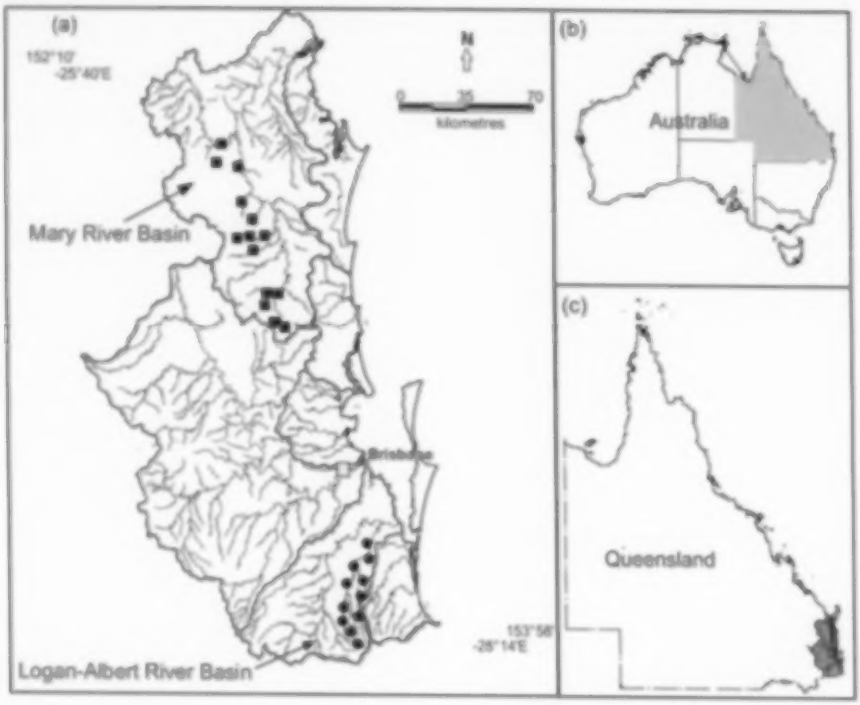
Fig. 1. (*a*) Location of the study sites in two river basins in southeastern Queensland. The insets (*b*) and (*c*) show the location of the study area in Queensland, Australia.



tionships between body length and mass for each species (Pusey et al. 2004). We calculated the following fish assemblage attributes for each site and sampling occasion: species composition (the presence or absence of species); species relative abundance (the percentage of the total number of individuals); and species relative biomass (the percentage of the total biomass of fish).

Environmental and hydrological descriptors

A set of the most ecologically relevant environmental and hydrological predictor variables were chosen from a larger number of candidate predictor variables derived from the literature and prior research in the study region (see Pusey et al. (2004) and references therein). Bivariate plots and Spearman's correlations among variables were used to identify and remove highly correlated variables. Spearman's correlation coefficients among the final set of predictor variables usually ranged between –0.5 and +0.5. Two landscape-scale variables and four local-scale variables were selected to describe environmental characteristics of the study sites (Table 1). The two landscape-scale variables (upstream catchment area and distance to the river mouth of each site) were estimated from 1:100 000 topographic maps using a digital planimeter. On each sampling occasion for each study site, the four local-scale environmental variables were measured or estimated using standard protocols described in Pusey et al. (2004). Mean wetted stream width, mean water velocity, and mean water depth were calculated from a series of 40- to 60-point measurements located randomly throughout the site. Mean riparian canopy cover was estimated from three site measurements using a spherical densometer.

We quantified antecedent flow conditions over three different time periods: long term (25 years), short term (the 4 months immediately preceding the time of sampling), and the preceding spawning season (September to March). The long-term hydrological record summarises conditions relating to disturbance regimes, aquatic habitat connectivity, overall habitat availability, and temporal variation in habitat structure and its spatial variability. We hypothesised that these factors were potentially important to fish via long-term temporal variation in individual species recruitment patterns and the frequency of local colonisation and extinction events. Short-term hydrological conditions indicate the presence of potential disturbance events prior to sampling, as well as the extent to which conditions at the time of sampling differ greatly from those in the preceding 4 months. The third group of flow attributes characterized spawning season conditions for the majority of fish species in southeastern Queensland (Pusey et al. 2004).

Six variables describing the long-term hydrological regime were calculated (Table 1). These included mean daily runoff (daily discharge standardised by upstream catchment area), variability (coefficient of variation) of daily flow, mean duration of zero flow events, and predictability of mean daily flow, minimum instantaneous flow, and maximum instantaneous flow in each month. Colwell's (1974) index (*P*) was used to quantify predictability in terms of a constancy factor (*C*) and a contingency factor (*M*), the sum

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Table 1. Means and 95% confidence intervals (CI) for environmental and hydrological variables used as predictors in the neural network models developed for the Mary River and tested in the Albert River.

		Mary River (training data)		Albert River (test data)	
Predictor variables	Code	Mean	95% CI	Mean	95% CI
Landscape-scale environment					
Catchment area upstream of site (km^2)	catarea	815.6	46.8, 1584.4	194.9	39.5, 350.2
Distance to river mouth (km)	distm	202.2	167.2, 237.1	77.0	57.8, 96.2
Long-term flow regime					
Mean daily runoff ($ML \cdot km^2$)	l_mdr	0.63	0.49, 0.76	0.83	0.73, 0.92
CV of daily flow	l_cvd	6.53	5.79, 7.27	5.81	5.17, 6.45
Predictability (*P*) mean daily flow (month)	l_pmndf	0.22	0.19, 0.25	0.15	0.14, 0.16
Predictability (*P*) minimum monthly flow	l_pmidf	0.16	0.13, 0.19	0.11	0.10, 0.13
Predictability (*P*) maximum monthly flow	l_pmxdf	0.57	0.52, 0.63	0.40	0.37, 0.44
Mean duration of zero flow events (days)	l_mdzf	12.0	5.6, 18.4	0	0, 0
Local-scale environment					
Mean riparian canopy cover (% shaded)	ripcov	44.8	40.8, 48.8	35.1	29.6, 40.6
Mean wetted stream width (m)	width	10.5	9.1, 11.8	8.3	7.5, 9.1
Mean water column depth (m)	avdepth	0.38	0.35, 0.41	0.40	0.36, 0.45
Mean water velocity ($m \cdot s^{-1}$)	avvel	0.14	0.12, 0.17	0.19	0.16, 0.21
Short-term flow history					
No. of zero flow days, 4 months before sample	s_zfd	18.6	13.5, 23.7	0	0, 0
No. of flow events greater than 1.67 annual return interval, 4 months before sample	s_hfe	0.44	0.34, 0.55	0.54	0.37, 0.70
Mean daily runoff, 4 months before sample ($ML \cdot km^2$)	s_mdr	0.35	0.28, 0.42	1.02	0.72, 1.32
CV daily flow, 4 months before sample	s_cvd	2.65	2.39, 2.91	2.01	1.72, 2.29
Mean daily runoff, spawning season before sample ($ML \cdot km^2$)	s_mdrsp	0.41	0.37, 0.45	0.51	0.45, 0.57
CV daily flow, spawning season before sample	s_cvdsp	4.09	3.84, 4.35	3.28	2.96, 3.60

Note: Variable abbreviations are used in Fig. 3. CV, coefficient of variation.

of which equals *P*. This index ranges from 0 (random) to 1 (totally predictable). We allocated discharge values to one of 11 flow classes with a central class of 20 times mean daily flow, using $\log_2$ classes (Marsh et al. 2003). We distinguish variability, which is insensitive to temporal pattern, from predictability, a measure of temporal uncertainty in periodic phenomena, and expect both to be ecologically important (Poff and Ward 1990; Pusey et al. 2000).

Short-term antecedent conditions (Table 1) were characterised by four variables: mean daily runoff, coefficient of variation of daily flow, number of zero flow days, and the number of high flow events (flow events greater than 1.67 year average recurrence interval) for the 4 months immediately preceding the time of sampling. We chose this high flow threshold as it approximates bankfull flow conditions and hence the flow magnitude yielding close to maximum water velocities, turbulence, shear stress, and stream power. The magnitude of the 1.67-year average recurrence interval flood was estimated from a 25-year hydrological record at each site using the annual duration series. The flood event independence criterion was defined as 7 days for these calculations. Lastly, we characterized hydrological conditions prevailing over the spawning period prior to sampling by estimating the mean daily runoff and coefficient of variation of daily flow (Table 1).

All flow variables for each study site were derived using 25 years (1974–1997) of daily discharge data simulated using integrated quantity and quality models (IQQM; Simons et al. 1996). IQQM, a tool used widely in Australia and elsewhere for water resource planning and assessment, can simulate flow volumes under various management scenarios including before water resource development, existing water use, and hypothetical future water demand. IQQM uses the Sacramento rainfall–runoff model based on rainfall and evaporation data for the generation of subcatchment runoff and operates on a continuous basis with a daily time step. IQQM parameters are determined by calibration against observed streamflow data (Simons et al. 1996; Merritt et al. 2003). IQQM for the Mary and Albert rivers were developed by the Queensland Department of Natural Resources and Water and were calibrated using daily flow data from 28 and 9 stream gauging stations in the Mary and Albert rivers, respectively (see Brizga et al. (2004, 2006) for further details on IQQM development and calibration). We used daily flow data simulated for existing water use (which was minimal for our study sites). Use of IQQM modelled flow data to estimate summary hydrological statistics for short temporal sequences (i.e., within-year) may be prone to greater error than when based on longer time series. We do not view this as being problematic in our study as all sampling sites were located within 20 km of stream gauges used to calibrate the IQQM developed for each river basin. Therefore, the temporal sequence of flows modelled by IQQM should accurately represent the existing pattern of flow as measured at the

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stream gauges. All flow variables were calculated using the river analysis package (Marsh et al. 2003).

Statistical analyses

Multiresponse artificial neural network

Species presence–absence, relative abundance, and relative biomass in the Mary River samples were modelled as a function of the 18 environmental and hydrological variables using a multiresponse artificial neural network (see Olden 2003; Olden et al. 2006*a*). In addition to the flexibility of neural networks to model multiple response variables, they are capable of modelling nonlinear associations with a variety of data types, require no specific assumptions concerning the distributional characteristics of the independent variables, and can accommodate interactions among predictor variables without any a priori specification (Bishop 1995). Neural networks have been shown to exhibit substantially higher predictive power (based on empirical and simulated data) when modelling nonlinear relationships compared with logistic regression, linear discriminant analysis, or classification trees (Olden and Jackson 2002).

We used feed-forward neural networks trained by the back-propagation algorithm to model spatial and temporal variation in three response variables: species presence or absence, relative abundance, and relative biomass. The architecture of these networks consisted of a single input, hidden, and output layers. The input layer contained one neuron for each of the environmental variables (18 in total). The number of neurons in the single hidden layer was chosen to minimize the trade-off between network bias and variance by comparing the performances of different cross-validated networks, with 2 to 50 hidden neurons (increasing by increments of 2), and choosing the number that produced the greatest external network performance. This resulted in the selection of 8, 9, and 14 hidden neurons for the modelling of species presence–absence, relative abundance, and relative biomass, respectively. The output layer contained one neuron for each fish species being modelled, representing either the probability of species presence–absence (decision threshold of 0.5), species relative abundance, or species relative biomass.

Model training involved the cross-entropy error function for species presence or absence (suitable for binary variables) and sums of squared error function for species relative abundance and biomass (suitable for continuous variables). Learning rate (η) and momentum (α) parameters (varying as a function of network error) were included during network training to ensure a high probability of global network convergence and a maximum of 1000 iterations for the back-propagation algorithm to determine the optimal axon weights. We refer the reader to Olden (2003) and Olden et al. (2006*a*) for more methodological details. The contributions of the environmental and hydrological variables in the neural networks were quantified by calculating the product of the input–hidden and hidden–output connection weights between each input neuron and output neuron and then summing the products across all hidden neurons. This approach is deemed the most appropriate as it has been shown to outperform other techniques for quantifying variable contributions in neural networks (Olden et al. 2004). All neural network analyses were conducted using computer macros written in the MatLab® (The MathWorks, Natick, Massachusetts) programming language.

Model validation and performance

Model validation was conducted in two ways. First, *n*-fold cross validation was used to generate model predictions and assess classification performance of the neural networks based on the Mary River data. This validation method excludes one observation, constructs the model with the remaining $n - 1$ observations, predicts the response of the excluded observation using this model, and repeats the procedure n times. Second, external validation was conducted by using the Mary River neural networks to predict the fish assemblages in the Albert River samples. This analysis provided an opportunity to assess the generality or transferability of the Mary River models to other river systems within the same biogeographic region in eastern Australia. Model performance for species presence–absence was assessed by calculating three metrics: overall classification performance (percentage of sites where the model correctly predicts species presence–absence), sensitivity (percentage of the sites where species presence was correctly predicted), and specificity (percentage of the sites where species absence was correctly predicted) (see Fielding and Bell 1997). Model performance for species relative abundance and biomass was assessed using Pearson's product–moment correlation coefficient (r) (a measure of prediction accuracy) and root mean square error (RMSE) (a measure of prediction precision). We assessed the statistical significance of species predictions following the protocols of Olden et al. (2002). Null distributions of correct classification rates (CCRs) for each species presence–absence were generated by randomly permuting the original observations among the stream sites, constructing an artificial neural network using the randomized data and the original environmental variables, and calculating the CCR (based on *n*-fold validation). This procedure was then repeated 9999 times and the significance level of the predictive model was calculated as the proportion of random CCRs (including the observed CCR) that were larger than or equal to the observed CCR. The randomization test was repeated for species relative abundance and biomass using the correlation coefficient (r). We assessed the overall performance of the neural network models by calculating the degree of concordance between predicted and observed fish assemblages (defined by species presence–absence, relative abundance, and biomass) using the Bray–Curtis similarity coefficient (Krebs 1999).

Results

Characteristics of the fish fauna and the riverine environment

Twenty-five fish species from 13 families were collected in the Mary River, 18 of which were also present in the Albert River (Table 2). Fourteen species, each representing greater than 1% of the relative abundance, were used for prediction of species relative abundance and biomass. Although fish assemblage characteristics varied between the two rivers, there were significant correlations among rivers in terms of species frequency of occurrence (Pearson's r =

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Table 2. Characteristics of the fish fauna in Mary River and Albert River.

		Mary River (training data)			Albert River (test data)		
Fish taxa	Common name	FOC	RA	RB	FOC	RA	RB
Anguillidae							
Anguilla reinhardtii Steindachner, 1867	Long-finned eel	86.1	5.60	43.78	98.6	10.06	51.84
A. australis Richardson, 1841	Short-finned eel	2.5	0.01	0.01	11.3	0.56	0.03
Retropinnidae							
Retropinna semoni (Weber, 1895)	Australian smelt	77.8	10.24	2.24	87.3	28.70	4.73
Plotosidae							
Tandanus tandanus Mitchell, 1838	Eel-tailed catfish	84.2	2.42	24.42	83.1	2.89	26.35
Neosilurus hyrtlii Steindachner, 1867	Hyrtl's tandan	7.0	0.05	0.27			
Atherinidae							
Craterocephalus marjoriae Whitley, 1948	Majorie's hardyhead	82.9	11.32	4.88	67.6	10.85	1.19
C. stercusmuscarum (Günther, 1867)	Fly-specked hardyhead	50.0	3.99	1.01			
Melanotaeniidae							
Melanotaenia duboulayi (Castelnau, 1878)	Duboulay's rainbowfish	88.6	10.70	6.14	80.3	21.64	5.51
Pseudomugilidae							
Pseudomugil signifer Kner, 1865	Pacific blue-eye	96.2	26.95	3.34	31.0	3.34	0.38
Synbranchidae							
Ophisternon sp. (undescribed)	Swamp eel	1.9	0.02	0.04			
Chandidae							
Ambassis agassizii Steindachner, 1867	Agassiz's glassfish	58.9	4.18	1.60	16.9	0.25	0.07
Percichthyidae							
Macquaria ambigua Richardson, 1845	Yellowbelly	0.6	0.02	0.58			
M. novemaculeata (Steindachner, 1866)	Australian bass	0.6	0.02	0.01	2.8	0.02	0.01
Nannoperca oxleyana Whitley, 1940	Oxleyan pygmy perch	0.6	0.01	0.01			
Terapontidae							
Leiopotherapon unicolor (Günther, 1859)	Spangled perch	29.7	0.59	1.86	11.3	0.13	0.56
Apogonidae							
Glossamia aprion (Richardson, 1842)	Mouth almighty	27.8	1.05	2.43			
Eleotridae							
Gobiomorphus australis (Krefft, 1864)	Striped gudgeon	1.9	0.01	0.10	36.6	5.30	5.88
Hypseleotris compressa (Krefft, 1864)	Empire gudgeon	14.6	0.78	0.39	14.1	1.69	0.32
H. galii (Ogilby, 1898)	Firetailed gudgeon	69.0	5.33	0.94	47.9	5.48	1.21
H. klunzingeri (Ogilby, 1898)	Western carp gudgeon	65.2	6.66	1.15	46.5	2.01	0.63

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Table 2 (*concluded*).

Fish taxa	Common name	Mary River (training data)			Albert River (test data)		
		FOC	RA	RB	FOC	RA	RB
Mogurnda adspersa (Castelnau, 1878)	Purple-spotted gudgeon	57.6	1.67	1.63			
Philypnodon sp. (undescribed)	Dwarf flathead gudgeon	43.0	0.72	0.26	16.9	0.41	0.09
P. grandiceps (Krefft, 1864)	Flathead gudgeon	41.1	0.87	0.71	19.7	0.49	0.37
Poeciliidae							
Gambusia holbrooki (Girard, 1859)*	Eastern mosquitofish	36.1	4.82	1.25	53.5	6.02	0.75
Xiphophorus helleri Heckel, 1848*	Swordtail	17.7	1.97	0.96	8.5	0.19	0.08
		Samples	Individuals	Biomass	Samples	Individuals	Biomass
	n =	158	68 815	712.6	71	20 697	131.4

Note: The frequency of occurrence (FOC), relative abundance (RA), and relative biomass (RB) of each species are shown for each river. These data are expressed as percentages of the total (n) number of samples, total number of individuals, and total biomass (kg) of fish collected in each river, respectively. Fish families are arranged in approximate phylogenetic order (after Pusey et al. 2004). An asterisk (*) indicates nonindigenous species.

0.74, $p < 0.001$), relative abundance ($r = 0.47$, $p < 0.05$), and relative biomass ($r = 0.95$, $p < 0.001$) (Table 2). The range of variation in environmental and hydrological conditions at the Mary River sites was generally similar to, or greater than, the range at Albert River sites (Table 1). This implies that fish should respond in similar ways to environmental and hydrological conditions in each river and therefore that a predictive model developed for the Mary River may have the capacity to predict fish assemblages in the Albert River. The major exception was that sites in the Albert River were situated relatively closer to the river mouth than sites in the Mary River, primarily because the Albert River catchment is smaller in size. The predictability of mean and maximum flows, and the incidence of zero flows, were lower in the Albert River. Mean daily runoff prior to sampling was usually higher and the variability of daily flows was usually lower in the Albert River than in the Mary River (Table 1).

Species-level model performance

The neural network model exhibited high success in predicting individual species presence or absence in the Mary River samples (Table 3). Mean correct classification rate was 87.3%, where 20 of the 25 species were correctly classified ($p < 0.05$) and all but one species (*Hypseleotris klunzingeri*) had correct classification rates exceeding 77%. Overall, the model was better able to correctly predict the absence of species than their presence (mean specificity = 76.2% and mean sensitivity = 60%), an expected result given the low frequency of occurrence of many species in the data set. The model had difficulty predicting the presence of rare species (i.e., low sensitivity) and the absence of some widespread species (low specificity). For example, those species present in less than 3% of samples were never correctly predicted (sensitivity values = 0%), and the five most widespread species (blue-eye (*Pseudomugil signifer*), rainbowfish (*Melanotaenia duboulayi*), eel (*Anguilla reinhardtii*), catfish (*Tandanus tandanus*), and hardyhead (*Craterocephalus marjoriae*)) present in more than 82% of samples had among the lowest specificity values (50% or less) (Table 3). When applied to the Albert River sites, the Mary River model did not perform as effectively. Although the mean correct classification rate across all 25 species was 73%, only 12 of the 25 species were correctly classified ($p < 0.05$). Mean specificity (56.8%) and sensitivity (57.0%) values were also lower for the Albert River data set than for the Mary River data set (Table 3).

The predictive model of species relative abundances was moderately accurate in the Mary River. The abundances of six of the 14 species modelled could be accurately predicted ($p < 0.05$), although correlations (Pearson's r values) between predicted and observed values were greater than 0.5 for nine of the 14 species examined (Table 3). The neural network based on species relative biomass was less accurate, however, with predictions of individual species biomass being significant for only two species and r values being generally low (Pearson's $r > 0.5$ in only 2 of 14 species). Predictions of species abundance and biomass data for Albert River were less accurate than for Mary River. The abundance and biomass of three species and of one species, respectively, were significantly predicted in the Albert River, but correlations between observed and expected values were less than 0.5 in all but one species comparison. No consistent pattern in the precision (as assessed by RMSE values) of species predictions was evident between rivers or assemblage data type (abundance versus biomass) (Table 3), and the predictive accuracy and precision appeared unrelated to the overall relative abundance or biomass of each species in the data set (Tables 2 and 3).

Assemblage-level model performance

Assessments at the assemblage level showed that the neural network predictions of fish species composition (based on presence–absence data) in the Mary River were highly concordant with observed composition. Mean Bray–Curtis similarity between predicted and observed species composition was 84.3%, and nine-tenths of samples contained fish assemblages that were greater than 70% similar to those predicted (Fig. 2). Model performance for assemblage estimates of relative abundance and biomass were lower, where mean

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Table 3. Model performance for predicting species presence–absence, relative abundance, and biomass in Mary River (training) and Albert River (test data).

	Mary River (training data)								Albert River (test data)							
		Presence–absence			Abundance		Biomass			Presence–absence			Abundance		Biomass	
Species	FOC	CC	SE	SP	*r*	RMSE	*r*	RMSE	FOC	CC	SE	SP	*r*	RMSE	*r*	RMSE
A. reinhardtii	86	87***	95	41	0.87***	0.07	0.30*	0.37	99	99***	100	0	0.31*	0.20	0.22	0.36
A. australis	3	98***	0	100					11	89	0	100				
R. semoni	78	81***	90	49	0.41	0.15	–0.07	0.07	87	87***	100	0	0.14	0.45	0.07	0.10
T. tandanus	84	86*	95	40	0.24	0.06	0.25	0.29	83	83	100	0	0.16	0.05	0.17	0.33
N. hyrtlii	7	94***	46	98					0	99***	—	99				
C. marjoriae	83	87***	95	44	0.46	0.15	0.25	0.09	68	55***	46	74	0.66***	0.13	0.20	0.06
C. stercusmuscarum	50	77***	77	77	0.51	0.08	0.43	0.03	0	56	—	56				
M. duboulayi	89	89	98	22	0.59*	0.11	0.72	0.07	80	80	100	0	0.12	0.32	0.03	0.12
P. signifer	96	98	100	50	0.58	0.22	0.21	0.08	31	31	100	0	–0.11	0.13	–0.17	0.06
Ophisternon sp.	2	98*	0	100					0	100***	—	100				
A. agassizii	59	82***	86	77	0.61*	0.06	0.48	0.04	17	51	58	49	–0.14	0.02	0.00	0.01
M. ambigua	1	99*	0	100					0	100***	—	100				
M. novemaculeata	1	99	0	100					3	97	0	100				
N. oxleyana	1	99*	0	100					0	100***	—	100				
L. unicolor	30	78	57	87					11	72***	50	75				
G. aprion	28	80**	57	89	0.75*	0.02	0.67*	0.07	0	63	—	63				
G. australis	2	98**	0	100					37	63	0	100				
H. compressa	15	87	39	96					14	86	10	98				
H. galii	69	84***	89	74	0.53	0.09	0.21	0.03	48	45	77	16	0.08	0.13	0.29***	0.04
H. klunzingeri	65	68*	80	46	0.61*	0.10	0.26	0.03	47	47	100	0	0.04	0.10	0.20	0.02
M. adspersa	58	77***	84	67	0.21	0.03	0.17	0.06	0	69***	—	69				
Philypnodon sp. A	43	83***	79	84					17	66**	33	73				
P. grandiceps	41	78***	68	85					20	58	57	58				
G. holbrooki	36	79***	75	81	0.38	0.12	–0.01	0.09	54	55***	95	9	0.18*	0.13	0.16	0.03
X. helleri	18	97***	89	99	0.60*	0.06	0.38	0.04	9	75***	0	82	–0.11	0.01	–0.12	0.04
Mean		87	60	76	0.53	0.09	0.30	0.10		73	57.0	57	0.12	0.15	0.09	0.11

Note: For the 25 species presence–absence model, reported values are species frequency of occurrence (FOC, percent of samples), percent correct classification rate (CC), sensitivity (SE, true positive classification rate), and specificity (SP, true negative classification rate). For the 14 species relative abundance and relative biomass models, reported values are Pearson's correlation coefficients (r) between predicted and observed values and the root mean square error (RMSE) of the predictions. Predictions of species presence–absence (CC), relative abundance, and relative biomass (r) that differ significantly from random are denoted by * ($p < 0.05$), ** ($p < 0.01$), or *** ($p < 0.005$).

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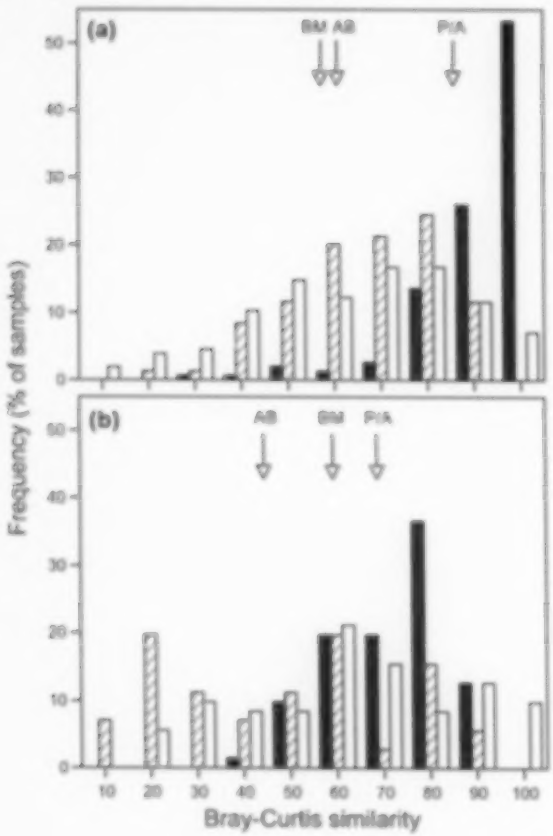
Fig. 2. Comparison of overall accuracy of artificial neural network (ANN) models to predict fish assemblages based on presence–absence (P/A, solid bars), relative abundance (AB, hatched bars), and relative biomass (BM, open bars) data for (*a*) Mary River (training data) and (*b*) Albert River (test data). Histograms are frequency distributions (percentage of samples) of Bray–Curtis similarities between fish assemblages predicted by ANN models and those observed using the same 14 species in each data set. Arrows represent the mean Bray–Curtis similarity.

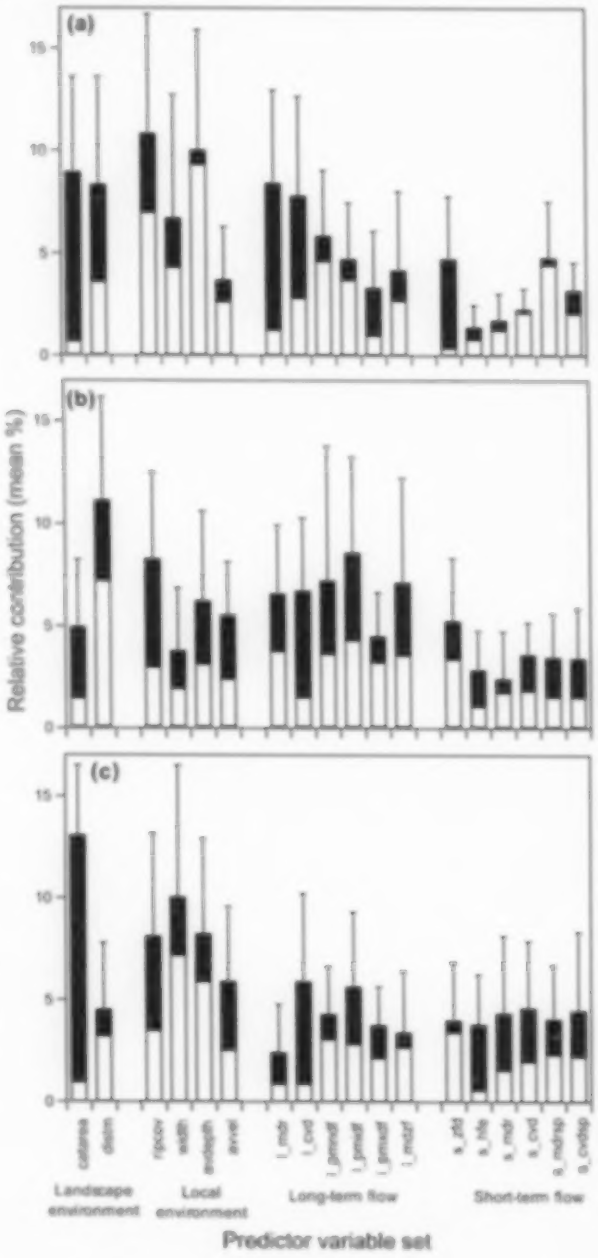


Bray–Curtis similarity between predicted and observed species assemblage structure was 62% and 59%, respectively. For both data sets, one-half of samples contained fish assemblages that were greater than 70% similar to predicted assemblages. In summary, a closer match between predicted and observed assemblages was observed for species presence–absence data than for species abundance or biomass data, the latter data being predicted with generally equivalent accuracy in the Mary River (Fig. 2). In all cases, model performance was greater for Mary River sites than for Albert River sites (Fig. 2).

Environmental and hydrological drivers of fish assemblage structure

Large- and local-scale environmental variables were important predictors of species occurrences in the Mary River

Fig. 3. Relative contributions of environmental and hydrological variables for predicting fish assemblages based on (*a*) presence–absence, (*b*) relative abundance, and (*c*) relative biomass data averaged (±1 standard deviation) across the 14 fish species common to each artificial neural network (ANN) model. Open portions of each bar represent the relative frequency with which each predictor variable had a positive influence on species predictions, and solid portions of each bar indicate the relative frequency of negative influences on species predictions. Codes are defined in Table 1.



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(Supplemental Table S1).[2] Catchment area and distance to river mouth were relatively important predictors (mean relative contribution = 8% and 9%, respectively) of the total variation in assemblage composition. Three of the four local environmental variables (riparian cover, stream width, and mean depth of channel) were also important predictors (>5%) of the total variation. Increasing catchment area and distance from river mouth typically had a negative influence on the predicted presence of a species, whereas the local-scale environmental variables usually had a positive effect (Supplemental Table S1).[2] Characteristics of the long-term flow regime were also important predictors of assemblage composition, particularly mean daily runoff, coefficient of variation of daily flow, the predictability (Colwell's *P*) of mean daily flow, and predictability of minimum instantaneous flow. Overall, short-term flow variables were comparatively less influential (mean relative contribution < 5%), except those describing mean daily runoff during the spawning season before sampling and the number of zero flow days during the 4 months before fish collection.

Comparison of variables for predicting species composition (Supplemental Table S1),[2] relative abundance (Supplemental Table S2),[2] and relative biomass (Supplemental Table S3)[2] across the 14 species common to each model (Fig. 3) revealed similar patterns in the major classes of important predictor variables. However, some variation in the importance of individual variables within each class of predictors was evident. Landscape environment, local environment, and long-term flow variables were comparatively more important than short-term flow variables in predicting each biological response data set. However, catchment area and stream width were stronger predictors of variation in species relative biomass than species composition and abundance (Fig. 3). The importance of long-term flow predictability was comparatively higher for species relative abundances in comparison with the other fish assemblage data sets. Furthermore, most aspects of short-term flow history assumed generally equal importance for species relative biomass in comparison with only one or two variables dominating within this class of predictors for species composition and relative abundances.

The magnitude and direction of connection weights in the neural network (Fig. 4) reveal nonlinear species–environment relationships. We highlight several examples in which strong variable interactions were important for model predictions of fish assemblage composition. Some of the linkages demonstrate the importance of interactions between long-term flow and local-scale habitat structure. For example, note the interaction between variability of daily flows (long-term) and water depth via hidden neuron G for eels (*A. reinhardtii*), smelt (*Retropinna semoni*), catfish (*T. tandanus*), rainbowfish (*M. duboulayi*), blue-eyes (*P. signifer*), and mouth almighty (*Glossamia aprion*). Increasing variability of daily flows decreased the probability of occurrence of these species, except in deeper sites. Landscape-scale factors (e.g., catchment area) were revealed by our model to influence species composition of assemblages via interactions with the predictability of minimum monthly flow (long-term) through hidden neuron F. Increased predictability of these minimum flows increased the probability of occurrence of three gudgeons (*H. galii* and *Philypnodon* spp.), except in large subcatchments. A synergistic interaction between coefficient of variation (CV) of daily flow (long-term), number of zero flow days (short-term), and stream width through hidden neuron D is evident for many species. Increasing variability of daily flow coupled with increasing duration of zero flows in wider streams decreased the probability of occurrence of glass perchlets (*Ambassis agassizii*) and three species of gudgeons (*H. galii* and *Philypnodon* spp.). We also found an interaction between two short-term flow variables (mean daily runoff during the spawning season with CV of daily flow during the spawning season) through hidden neuron B for the gudgeon *H. klunzingeri* and the nonindigenous species *Gambusia holbrooki*. Increasing mean daily runoff during the spawning season increased the probability of occurrence of these species, except when runoff was highly variable.

Discussion

This study aimed to gain insight into the relative influence of multiscaled environmental and hydrological features of the riverine landscape on different properties of fish assemblages and to evaluate the generality of these relationships among nearby catchments. Multiscaled approaches such as ours should be well suited to the study of riverine fish. Compared with other aquatic biota, fish are long-lived and mobile and so may occupy a variety of habitats and hence may encounter a range of environmental conditions throughout their life span (Fausch et al. 2002). However, these ecological factors, together with the complex hierarchical linkages evident in natural river ecosystems, can make it difficult to develop accurate predictive models and to isolate the importance of individual and interacting environmental controls on fish assemblages (Rose 2000).

The multispecies model for the Mary River provided accurate predictions of individual species occurrences (high mean correct classification rate) and assemblage composition (high similarity between predicted and observed assemblages). The important landscape- and local-scale environmental factors identified by this model are well-established correlates of, or are related mechanistically to, species distributions and fish assemblage composition in many river systems (Jackson et al. 2001). Attributes of the long-term flow regime were also important predictors of assemblage composition and likely govern the temporal availability and predictability of suitable habitat for various fish species. Long-term flow conditions also determine the spatial variability and connectivity of habitats that, in turn, influence local colonisation and extinction events (Magoulick and Kobza 2003; Matthews and Marsh-Matthews 2003; Scheurer et al. 2003).

The neural network provided significant insight into the important interactions among environmental and hydro-

[2] Supplementary data for this article are available on the journal Web site (http://cjfas.nrc.ca) or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Building M-55, 1200 Montreal Road, Ottawa, ON K1A 0R6, Canada. DUD 5216. For more information on obtaining material refer to http://cisti-icist.nrc-cnrc.gc.ca/irm/unpub_e.shtml.

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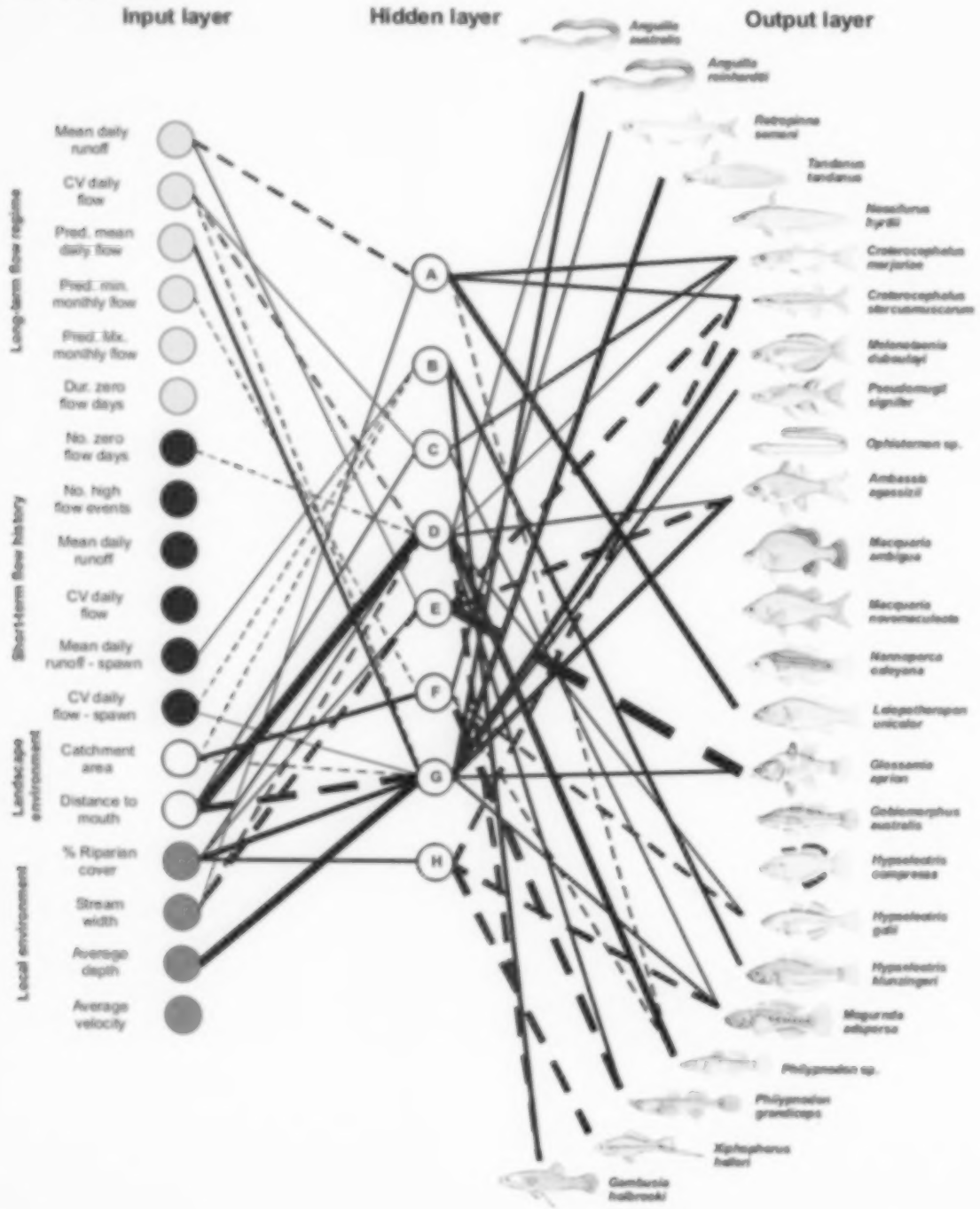
Fig. 4. Neural interpretation diagram showing linkages between environmental and hydrological variables used for predicting fish species composition in Mary River. Line thickness is proportional to the magnitude of the axon connection weight, and line type indicates the direction of the interaction between neurons: solid line, connections are positive (excitators); broken line, connections are negative (inhibitors). Only statistically significant connection weights are presented ($p < 0.05$). Individual species with no output connections were not accurately predicted. Multiplication of the two connection weight directions (positive or negative) indicates the effect that each input variable has on the response variable (fish). Input variables with larger connection weights are more important in predicting the output compared with variables with smaller weights. CV, coefficient of variation; Pred., predictability; Min., minimum; Mx., maximum; Dur., duration (mean).



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logical factors and into the potential mechanisms likely to influence variation in fish assemblage composition. The importance of interactions between variability of daily flows (long-term) and water depth identified for some fish species suggests that deeper locations are more effectively buffered against changes in habitat structure and quality associated with varying water levels than are shallow sites. The interaction between landscape-scale factors and long-term flow predictability suggests that species of gudgeons with benthic habits (Pusey et al. 2004) were more likely to occur at sites in smaller catchments if minimum flows were sufficiently predictable to ensure the availability of suitable benthic habitat over the long term. Our model also revealed the potential for aspects of the short-term flow history to influence the composition of fish assemblages. For example, an increased probability of fish stranding in wider streams with fluctuating water levels is suggested for some species by the negative influence of variable flow conditions interspersed with extended dry periods. In addition, increasing mean daily runoff during the spawning season before sampling increased the probability of occurrence of two species, except when runoff during this period was highly variable; this implies an adverse effect on their capacity to either spawn successfully or to recruit to larger sizes, or both. These positive and negative interactions collectively highlight the importance of long-term flow variables and local-scale habitat characteristics such as stream width and depth, enhanced or ameliorated by a particular catchment setting and recent flow conditions. These relationships are consistent with our knowledge of the ecology of fish assemblages in the region (Pusey et al. (2004) and references therein).

Comparison of model performances for the Mary River showed that species presence–absence was more predictable than both relative abundance and relative biomass. This scale-dependent relationship between environmental drivers and different assemblage response variables is expected (see Rahel 1990). Species presence–absence is a less "noisy" signal and less prone to spatial and temporal variation as it involves a local extinction process, not just a shift in abundance or biomass (Strange et al. 1992; Milner et al. 1993; Poff and Allan 1995). Indeed, in our study systems, spatial and temporal variation in fine-grained fish assemblage responses such as abundance and biomass data is much greater than for the coarser-grained measure of fish species presence or absence (Kennard 2005). Hence, it is not surprising that our models more accurately predicted species presence–absence.

The relative importance of the major classes of environmental and hydrological predictor variables was broadly similar for models predicting species occurrences, abundances, and biomass in the Mary River. That short-term flow variables played a comparatively small predictive role was contrary to our expectations, particularly for abundance and biomass data. The recent history of hydrological events has been shown elsewhere to be an important driver of fish abundance patterns via recruitment processes (e.g., Schlosser 1985; King et al. 2003). However, the dynamics of recruitment processes in fishes are highly complex, with environmental and biologically driven variation in abundances of all life stages contributing substantially to variability in final abundance (Bradford 1992). It is possible that the variables we used to quantify antecedent flow conditions were too coarse to represent flow-related changes in factors contributing to recruitment variation. Such factors could include the suitability and temporal stability of spawning sites, habitat condition, and food resource availability for larvae or the sequence of habitats and conditions necessary for juvenile growth and survival. Similarly, the connectivity of habitats in time and space could also be important, but the surrogate indicators we used, such as number of zero flow days or duration of zero flow days before sampling, may not represent connectivity effectively (Scheurer et al. 2003). In addition, our short-term flow predictors were unlikely to capture the influence of flow variation on biotic processes such as predator–prey relationships and competitive interactions among species that, in turn, can have significant effects on fish assemblage structure (Jackson et al. 2001). Furthermore, because we did not attempt to distinguish the independent explanatory effects of each set of environmental and hydrological drivers, the importance of short-term flow variation may have been obscured by covarying predictors at other spatial and temporal scales (although we avoided using highly correlated variables). It would therefore be instructive to compare the results of the present study with those of other methods that can uncover the unique contribution of different variable sets, including variance partitioning methods (e.g., Magalhães et al. 2002; Pont et al. 2005), or to analyze residuals from each stage of a hierarchical analysis (e.g., Olden et al. 2006*b*).

Evaluation of the transferability of our predictive models showed that the Mary River model based on species presence–absence data more accurately represented species–environment relationships in the Albert River than did models based on species abundances or biomass. This result suggests that although the landscape-scale features that drive the distribution of individual fish species are similar between these rivers, the particular interactions between the influence of landscape, hydrology, and local habitat features that collectively determine abundance and biomass differ between the two rivers. Although the importance of knowing that particular models are transferable is well recognised (Mac Nally 2002), previous modelling work with stream fishes has illustrated that model transferability can vary greatly depending on circumstances. In general, models constructed for one basin rarely perform as well in another basin (e.g., Fausch et al. 1988; Shirvell 1989; Leftwich et al. 1997). Our results support this earlier work but clearly show that model transferability depends on the type of biological response variable modelled. Model transferability may be compromised if the environmental factors that limit species distributions are not included or retained in the models or if they differ between catchments (Leftwich et al. 1997). Variation in species prevalence (i.e., frequency of occurrence) between systems can also affect spatial transferability of predictive models (Mac Nally 2002; Olden et al. 2002), and this may apply to abundance and biomass data as well. These issues highlight the need for a better understanding of the factors responsible for shaping individual species distributions and assemblage structure before catchment-specific models can be applied more broadly.

In conclusion, improved knowledge of ecological responses to the natural and human-altered characteristics of river flow regimes is urgently required to inform water resource man-

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agement (Arthington et al. 2006; Dudgeon et al. 2006). The demands facing managers as they attempt to allocate limited freshwater resources are escalating and exacerbated by limits in our quantitative understanding of how aquatic ecosystems, and their biological communities, respond to environmental conditions (Allan 2004). The complexity and nested arrangement of environmental factors shaping lotic communities makes the construction of useful predictive models a challenging task. Yet, we were able to model both the composition and structure (abundance and biomass) of fish assemblages and to incorporate all species comprising the assemblage simultaneously within a single integrated modelling process. This multispecies approach increases our capacity to detect and synthesise complex biota–environment relationships (Ferrier and Guisan 2006; Olden et al. 2006*a*). Predictive modeling studies such as ours contribute to a fundamental understanding of river ecosystem functioning in terms of identifying important interactions between flow regime and habitat at local to landscape scales, their effects on fish assemblage structure, and the generality of these relationships.

Acknowledgments

This study was funded by the Cooperative Research Centre for Freshwater Ecology, Land and Water Australia (formerly Land and Water Research and Development Corporation) and the Australian Rivers Institute. We thank Steve Mackay and Darren Renouf for assistance with field sampling. We appreciate the detailed comments by several anonymous reviewers, which substantially improved this paper. This research project was approved by the Griffith University Ethics Committee for Experimentation on Animals and the applied research protocols used were conducted in accordance with the requirements of this Committee.

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Physiological consequences of "premature freshwater return" for wild sea-run brown trout (*Salmo trutta*) postsmolts infested with sea lice (*Lepeophtheirus salmonis*)

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Abstract: The physiological consequences of "premature migratory return" to freshwater for wild sea-run brown trout (*Salmo trutta*) smolts infested with sea lice (*Lepeophtheirus salmonis*) were investigated in the laboratory. Osmoregulatory, metabolic, and stress markers were analysed in order to assess the potential consequences of transfer to freshwater, 19 days after the challenge with *L. salmonis*. Infestation intensity was significantly reduced following transfer to freshwater, and mortality rates were markedly higher in infested fish maintained in seawater vs. fish that were transferred to freshwater. Significant sea lice effects, consistent across a number of physiological markers, were apparent once *L. salmonis* developed to the mobile stages. Plasma chloride, lactate, and cortisol all were significantly higher than control values, and liver glycogen concentration was significantly reduced in infested fish in seawater. After return to freshwater, these physiological measures returned to control levels, but significant lice effects persisted for fish maintained in seawater. Premature return of infested sea-run brown trout to freshwater does, therefore, confer significant short-term physiological benefits across a range of osmoregulatory, metabolic, and stress markers.

Résumé : Nous avons étudié en laboratoire les conséquences physiologiques d'un « retour prématuré de migration » en eau douce chez des saumoneaux de truites brunes anadromes (*Salmo trutta*) sauvages et infestées de *Lepeophtheirus salmonis*. Nous avons analysé des marqueurs de l'osmorégulation, du métabolisme et du stress afin de déterminer les conséquences potentielles d'un transfert en eau douce, 19 jours après une confrontation avec *L. salmonis*. L'intensité de l'infestation est significativement réduite après le transfert en eau douce; les taux de mortalité sont nettement plus élevés chez les poissons infestés maintenus en eau salée par rapport aux poissons transférés en eau douce. Des effets significatifs des poux de mer, retrouvés dans plusieurs marqueurs physiologiques, apparaissent une fois que *L. salmonis* atteint les stades mobiles. Chez les poissons infestés en eau de mer, les concentrations plasmatiques de chlorures, de lactate et de cortisol sont toutes significativement plus élevées que chez les témoins et la concentration de glycogène du foie est significativement réduite. Après un retour en eau douce, les mesures physiologiques reviennent aux valeurs témoins, alors que des effets significatifs des poux persistent chez les poissons retenus en eau de mer. Un retour prématuré en eau douce des truites brunes anadromes infestées procure ainsi des bénéfices physiologiques qui sont apparents dans une gamme de marqueurs de l'osmorégulation, du métabolisme et du stress.

[Traduit par la Rédaction]

Received 11 October 2006. Accepted 24 March 2007. Published on the NRC Research Press Web site at cjfas.nrc.ca on 29 September 2007.
J19592

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 doi:10.1139/F07-107 © 2007 NRC Canada

Introduction

In northwestern Europe, the sea-run brown trout (*Salmo trutta*, hereafter referred to as sea trout) is the anadromous form of the freshwater brown trout. There has been considerable recent concern over the catastrophic decline of certain sea trout stocks in Ireland, Scotland, and Norway, which appears to be attributable primarily to increased marine mortality (Anonymous 1993; Northcott and Walker 1996; Butler 2002). Unlike salmon, which undertake extensive oceanic migrations (Hansen and Jacobsen 2000), sea trout remain in nearshore waters throughout their marine residence (Klemetsen et al. 2003) and are persistently subject to coastal stressors and sources of mortality. Caligid sea lice infestations can debilitate or kill the host salmonid (Pike and Wadsworth 2000), and there now is strong circumstantial evidence that sea lice derived from aquaculture pens have the potential to impact wild salmonids (Butler 2002; McKibben and Hay 2004; Penston et al. 2004).

Coincident with the first recognition of stock crashes were reports of "premature return" of postsmolt sea trout within only a few weeks of their first migration to sea. These fish typically bore heavy infestations of largely juvenile (chalimus), sessile stages of sea lice (Tully and Whelan 1993; Tully et al. 1993; MacKenzie et al. 1998). Despite large sample sizes and observations of high abundances of juvenile sea lice stages on infested fish, Tully et al. (1993) failed to capture juvenile sea trout bearing mobile, adult sea lice and concluded that any such host fish probably would have succumbed to infestation.

The copepod sea lice *Lepeophtheirus salmonis* (Krøyer) and *Caligus elongatus* Nordmann are the primary ectoparasites of farmed and wild salmonids (Pike and Wadsworth 2000), and their abundances can be high on return-migrant adult salmon (*Salmo salar*). Sea lice feed on the host epidermis and blood, and grazing by mobile preadult and adult sea lice can result in severe skin abrasion (Bjørn and Finstad 1998; Pike and Wadsworth 2000). This can be fatal to hatchery-reared juvenile sea trout and salmon (Grimnes and Jakobsen 1996; Bjørn and Finstad 1997; Dawson et al. 1998). High abundances of sea lice on small juvenile salmonids induce obvious host stress and osmoregulatory imbalance, and these effects are especially intense when the lice develop to the mobile preadult and adult stages (Bjørn and Finstad 1997; Dawson et al. 1998; Wells et al. 2006). However, for wild one-sea-winter adult salmon captured on their migratory return to home waters, the abundances of the mobile stages of either sea lice species can exceed 100 on individual hosts (Todd et al. 2006). Such high abundances of sea lice appear not to be detrimental to the host adult salmon, perhaps because of their size (weight range 1.1–4.6 kg), and there is no significant correlation between infestation intensity and individual host condition factor (Todd et al. 2006).

Empirical data on the causes and consequences of premature return to freshwater (FW) for wild sea trout postsmolts are limited. Caligids are marine parasites and are readily lost from the host fish within a few hours or days of migration to brackish or FW (McLean et al. 1990). However, there are reports of clear host species differences in retention of caligids; for example, *L. salmonis* infesting Arctic char (*Salvelinus alpinus*) can survive for up to 3 weeks in FW (Finstad et al. 1995). Premature migratory return of sea lice infested sea trout postsmolts has been interpreted as an adaptive response by the host to osmoregulatory dysfunction in seawater (SW) (Birkeland 1996; Bjørn et al. 2001), but detailed physiological appraisal of this hypothesis currently is lacking. Indirect evidence that a link might exist between the physiological effects of sea lice infestation and premature return is provided by analyses of swimming performance of sea lice infested juvenile Atlantic salmon, which improved upon transfer to FW (Wagner et al. 2004). Although early return of sea trout to FW may allow short-term recovery from sea lice infestation, this benefit must be balanced against long-term health consequences, including minor osmoregulatory disturbances (Birkeland 1996; Bjørn et al. 2001). Moreover, growth opportunities and thereby future fecundity of individuals may be compromised by an abbreviated migration to sea. The primary objective of this study therefore was to assess, under controlled laboratory conditions and using a comprehensive suite of physiological markers, the consequences and potential benefits of premature return to FW for sea lice infested wild postsmolt sea trout.

Materials and methods

Wild sea trout smolts in FW were trapped from the Hals River, Altafjord, Norway (72°2′N, 22°5′E), over the period 24–28 June 2004 and transferred to tanks in the adjacent Talvik Research Station. Smolts were allocated randomly to experimental groups in duplicate tanks and maintained in FW under natural photoperiod (24 h daylight) until transfer to SW (33‰) on 29 June. The initial stocking density was 38 fish·tank^{-1} (total = 304; mean weight 77.8 g), a density and water volume per fish that would not be expected to induce stress (Einarsdottir and Nilssen 1996). There were four experimental groups, which were assigned to duplicate tanks: control SW, noninfested fish maintained throughout in SW; infested SW, sea lice infested fish maintained throughout in SW; control SW–FW, noninfested fish maintained in SW until 19 days after infestation (dpi) then returned to FW; and infested SW–FW, sea lice infested fish maintained in SW until 19 dpi then returned to FW. Six 800 L tanks and two 150 L tanks were available. To ensure that the sea lice infestations occurred in identical (800 L) duplicate tanks, one 150 L and one 800 L tank were each used as the duplicates for the SW and SW–FW control groups. The remaining 800 L tanks were allocated randomly to experimental groups. Fish were fed to satiation (based on a feeding rate of ~2% body weight·day^{-1}) on a combined diet of chopped squid and commercial fish food pellets, mixed with cod roe to encourage feeding. The mean aquarium water temperature was 14 ± 0.1 °C.

Ovigerous female *L. salmonis* were cultured at the Marine Institute, Bergen, as described previously (Bjørn and Finstad 1998). Infective copepodids (2 to 4 days old) were transported by air to Talvik in flasks of cooled SW and immediately used to infest the fish. The total number of larvae was estimated from five replicate subsample counts. At infestation, the volume of water in the experimental tanks was reduced to 100 L and the water supply was turned off. The water was continuously aerated during infestation to

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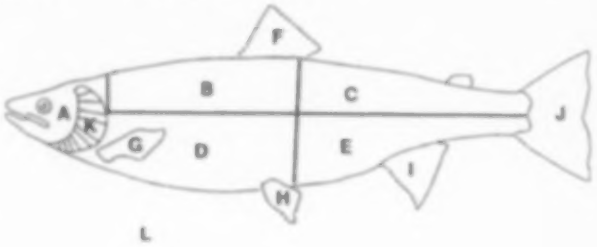
maintain circulation in the tanks. Copepodids were introduced in appropriate numbers (twice the desired infestation intensity per fish) to achieve an infestation intensity of ~50 *L. salmonis*·fish^{-1}. Previous experience (Wells et al. 2006) had shown such an intensity to be likely to cause sublethal stress responses. The remaining control tanks were treated in an identical manner, but no copepodid larvae were added. Infestation progressed for 4 h in darkness (Johnson and Albright 1991*a*), after which the water supply was reinstated to all tanks and the normal light regime was restored.

Control samples of fish were taken from all tanks on the day before sea lice infestation (designated as 0 dpi). Subsequently, all tanks were sampled at 7, 14, 21, and 28 dpi. Fish were individually netted and removed from the experimental tanks, taking care to minimise disturbance to the remaining fish, and anaesthetised in 2-phenoxyethanol (2.5 mL·L^{-1}) using separate containers for individual fish. Throughout the experiment, fish were never repeat-netted or handled. The anaesthetic was passed through a 40 µm filter to collect dislodged sea lice for classification. Five fish from each tank (10 fish for each sea lice intensity) were removed at each sampling date (except for the infested SW group at 28 and 35 dpi, owing to mortalities), and tissues were collected within 15 min. Blood samples (0.5 mL) were taken from the caudal blood sinus within 1 min of sacrifice, added to 50 µL 15% ammonium EDTA (ethylenediaminetetraacetic acid) and processed immediately (see below). Each fish was then opened along the midventral line, and the liver was removed and freeze-clamped in liquid nitrogen. Frozen livers were placed in preweighed vials and stored at –20 °C until analysis. Carcasses were placed in individual plastic bags and stored at 4 °C or –20 °C for later examination for sea lice. Fish were dissected on paper towelling, which also was bagged and subsequently examined for dislodged sea lice. The fork length and weight of all fish were recorded and expressed as the composite condition factor, Fulton's *K* (weight in grams × (length in centimetres)$^{-3}$ × 100). Ratio condition factors are sensitive to isometric variation and departures from the cube relationship, but the size range of experimental fish was narrow and Fulton's *K* has been shown to have a high correlation with whole-fish lipid content (Sutton et al. 2000; Neff and Cargnelli 2004).

Plasma was separated by centrifugation at 13 000 r·min^{-1} (1 r = 2π rad) for 1 min and frozen at –80 °C until analysis. Samples were transferred on dry ice to St. Andrews and Nijmegen for later analysis. Plasma chloride was measured by colorimetric titration (Chloride analyser 925; Corning Limited, Schiplo-rijk, the Netherlands). Plasma glucose concentrations were determined in duplicate using a commercially available kit (GL 2623; Randox Laboratories Ltd., Crumlin, Co. Antrim, UK), and absorbance was read at 490 nm. Plasma lactate concentrations were determined in duplicate using commercially available lactate reagent (LC 2389; Randox Laboratories Ltd.) and read at 540 nm. Both of the latter assays were modified for use in 96-well plates and a plate reader (Biotech Instruments Inc., Winooski, Vermont). Liver glycogen was assessed following hydrolysation by amyloglucosidase (Keppler and Decker 1974). Plasma cortisol was measured by radioimmunoassay (RIA) (van Anholt et al. 2003).

Fig. 1. The 11 zones on the body of the infested sea-run brown trout (*Salmo trutta*) smolts examined for sea lice (Bjørn and Finstad 1998): (A) head and operculum; (B) anterior dorsal area of the body above the lateral line from the head to the posterior end of the dorsal fin; (C) posterior dorsal area of the body above the lateral line from the posterior end of the dorsal fin to the caudal fin; (D) anterior ventral area of the body below the lateral line from the head to the posterior end of the dorsal fin; (E) posterior ventral area of the body below the lateral line from the posterior end of the dorsal fin to the caudal fin; (F) dorsal fin; (G) pectoral fin; (H) ventral fin; (I) anal fin; (J) caudal fin; (K) gills. The anaesthetic solutions, plastic storage bags, and paper towelling on which dissection took place were also examined and are shown as zone L.



The body surface, fins, and gills were separated analytically into 11 zones as previously described (Bjørn and Finstad 1998) (Fig. 1). These zones on all infested fish were examined for sea lice, and the frequencies of each developmental stage were recorded. Larval sea lice at chalimus stages 1 and 2 and chalimus stages 3 and 4 were pooled into two groups, termed C1 and C3, respectively. *Lepeophtheirus salmonis* stages were classified and their sex was determined according to Johnson and Albright (1991*b*) and Schram (1993).

Data analysis

The number of sea lice, fish length, weight, condition factor, treatment group, and days after infestation were considered as candidate variables in each model, in addition to interaction terms involving sea lice, days after infestation, and group. The data also were inspected for significant sampling order and tank effects. Length and weight were too highly correlated to include simultaneously in the models; these had variance inflation factors (Fox and Monette 1992) of 19.80 and 21.34, respectively, and because length resulted in better fitting models overall, this was chosen instead of weight.

Generalized additive models (GAMs) were used to model the nonlinearities between the covariates and response variables. Specifically, cubic regression splines were fitted within a linear model framework, and the necessity of these nonlinearities (as compared with their linear alternatives) was assessed using *F* tests. Interaction effects involving time, sea lice, and group were also considered in each model because the treatments given to each group and the effects of sea lice on the fish were closely related to time.

For models in which sea lice was retained as a variable, it was important to obtain group-specific measures of average sea lice numbers across time. For example, it was clear that FW re-entry resulted in sea lice detachment and that these

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fish had markedly fewer lice than were originally attached. This led to the group-specific modelling of average sea lice numbers across time, using a generalized nonlinear quasi-poisson model. The tank-specific sea lice loadings resulting from this model at each time point then were used to generate model predictions for each physiological marker; this ensured that a realistic number of sea lice at each time point was used for each model. Mean values of model covariates were used to generate these predictions.

A tank identifier was included in the model to account for any tank-specific effects because average sea lice numbers were significantly different between the two infested SW tanks and the two infested SW–FW tanks. Lice predictions based on this model were then averaged across the tanks at each time point, for both the infested SW and infested SW–FW groups. This resulted in slightly higher sea lice numbers for fish in the infested SW–FW group. These results then were used to predict levels of liver glycogen and plasma chloride, glucose, and lactate across time, as well as plasma cortisol at 14 dpi (Figs. 2*a*–2*d*). The data also were checked for possible handling stress related effects by examining the sequence of sampling individual fish on each occasion. No significant sequence effects were identified.

For each response variable, predictions were obtained for each time point based on the experimental group and the relevant predicted number of sea lice for that group at that time. Other covariates included in the model were fixed at their mean across days after infestation, except for plasma cortisol, which was considered at each time point. In this analysis, nonoverlapping intervals are considered to represent significant differences between underlying means for each time-specific group, i.e., intervals seen to overlap even in part are not considered to represent significant differences between group means. It should also be noted that these intervals were not adjusted for pairwise comparisons.

Models for each physiological marker were selected using Akaike's information criterion (AIC) scores (Akaike 1973) and p values resulting from F tests and likelihood ratio tests (Fox 1997). Typically, a preliminary model was chosen using the AIC score, followed by the omission of nonsignificant covariates at the 5% level; departures from this procedure are mentioned specifically (see Results). All models were fitted initially assuming normally distributed, independent errors with constant error variance; these assumptions were all assessed visually and supplemented by tests of significance. Specifically, Breusch–Pagan, Durbin–Watson, and Shapiro–Wilk tests were used for model residuals (Breusch and Pagan 1979; Royston 1982; Fox 1997). Any models that were found to have nonconstant error variance but exhibited symmetry were fitted using a generalised least squares framework, allowing a more appropriate mean–variance relationship. Models with response data bounded by zero were modelled using a generalized linear model with Gamma errors, as were models exhibiting nonnormal errors and nonconstant error variance.

Results

We were successful in acclimating wild sea trout smolts to tank conditions in a relatively short period of time. This was achieved by minimizing disturbance to the fish and optimizing water flow rate and by an intensive period of hand-feeding, particularly at the start of the experiment. Fish in all experimental tanks were feeding well within 1 week of capture and before the experiment commenced, but from 14 to 21 dpi onwards, fish in both infested groups showed a marked decrease in feeding. Although the feeding activity of the fish in the infested SW–FW tanks recovered somewhat following return to FW, throughout the remainder of the experiment the food intake of both infested groups remained lower than that of the control groups.

Development of *L. salmonis*

Lepeophtheirus salmonis numbers and developmental stages (Table 1) were assessed for sampled fish at each experimental time point (7, 14, 21, 28, and 35 dpi). Sea lice on mortalities were also counted, allowing the recording of developmental data and the determination of the first appearance of *L. salmonis* stages between sampling days. Mobile, preadult *L. salmonis* were observed from 17 dpi onwards. Prevalence of *L. salmonis* was 100% on each sampling occasion, with the exception of fish that had been returned to FW at 19 dpi (Table 2). Prevalence in these latter groups was 50% and 30% at 28 and 35 dpi, respectively, and the infestation intensity was greatly reduced in comparison with the infested SW groups. It is assumed, however, that noninfested fish in these experimental groups had been infested earlier in the SW phase of the experiment. Only copepodids and early chalimus stages (C1) were found at 7 dpi. One week later, the majority of *L. salmonis* had moulted to the late chalimus stages. By 21 dpi, most of the sea lice were at the preadult stage, with 50% at female preadult 1 and 35% at male preadult 2. Fifty percent of the *L. salmonis* were adult males by 28 dpi, with the majority of the remaining sea lice at preadult 2 – adult female stages. At 35 dpi, the lice were 50% adult males, 19% adult females, and 30% gravid females.

The frequency distribution of *L. salmonis* on the body surface of sampled fish is provided (Table 3). At 7 and 14 dpi, when almost all sea lice were at the chalimus stages (Table 1), the majority of sea lice were attached to the fins. Once most *L. salmonis* attained the mobile stage (21 dpi onwards), the majority were found on the body surface, but a substantial proportion was also found dislodged in the anaesthetic solution. Mean *L. salmonis* intensity and group-specific models of average sea lice numbers across time (which were used to generate model predictions for each physiological measure) are shown (Tables 3 and 4, respectively). It is clear that the infestation intensities exceeded the stress threshold of 13 *L. salmonis*·fish^{-1} (Wells et al. 2006) but were higher than intended. In addition, and despite efforts to ensure comparability of infestation intensities, there were clear tank effects within treatments.

Mortalities

There was a single fish mortality in the control SW group and six mortalities in the control SW–FW group. There were 13 mortalities in the infested SW–FW group, nine of which occurred prior to return to FW at 19 dpi. There were 46 mortalities in the infested SW group, and the rate of mortality increased rapidly once *L. salmonis*

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Table 1. Percentage frequency and first appearance of the major developmental stages of *Lepeophtheirus salmonis*.

	Stage and frequency									
Days after infestation	Cop	C1	C3	PAM1	PAM2	PAF1	PAF2	AM	AF	GF
7	9	91								
14		1	99							
21			2	11	35	50	3			
28					1	4	27	50	18	
35								50	19	30
1st appearance	—	7	10	17	17	17	17	22	27	35

Note: Infested fish (n = 20) were sampled at 3, 7, 14, 21, and 28 days after infestation (dpi). Stages are designated as follows: Cop, copepodid; C1, first and second chalimus stages combined; C3, third and fourth chalimus stages combined; PAM1, first preadult male; PAF1, first preadult female; PAM2, second preadult male; PAF2, second preadult female; AM, adult male; AF, adult female; GF, gravid female.

Table 2. Mean *Lepeophtheirus salmonis* intensity and prevalence in the two infested groups (n = 5 on each sampling occasion).

		Mean *L. salmonis* intensity		
Group	Days after infestation	Tank 1	Tank 2	Prevalence
SW infested	7	43.6 (14.6, 11.0)	78.5 (18.9, 15.3)	100
	14	46.5 (7.3, 6.3)	112.6 (14.2, 12.6)	100
	21	71.3 (7.2, 6.6)	127.5 (10.0, 9.3)	100
	28	42.4 (5.6, 5.0)	56.3 (2.9, 2.8)	100
	35	33.1 (4.2, 3.7)	—	100
SW–FW infested	7	52.5 (13.5, 10.8)	130.2 (23.2, 19.7)	100
	14	72.0 (12.4, 10.6)	128.2 (13.8, 12.4)	100
	21	39.3 (8.2, 6.8)	114.8 (13.3, 11.9)	100
	28	0.2 (0.3, 0.2)	2.8 (1.6, 1.1)	50
	35	0.0 (-)	3.4 (5.2, 2.4)	30

Note: Values are antilogged means with upper and lower standard errors shown in parenthesis. SW, seawater; FW, freshwater.

Table 3. Frequency distribution (%) of *Lepeophtheirus salmonis* on the body surface of sampled fish.

	Body surface					Fins					Gills	Anaesthetic
Days after infestation	A	B	C	D	E	F	G	H	I	J	K	L
7	3.3	0.7	3.6	2.8	2.5	27.0	14.7	11.3	7.3	13.9	5.7	7.1
14	5.2	1.5	6.2	4.6	4.6	28.1	16.4	7.8	5.4	15.7	3.2	1.3
21	11.8	17.5	20.4	6.0	12.5	1.7	0.5	0.3	0.2	1.5	1.5	26.3
28	9.7	14.7	24.4	1.3	9.7	0.8	0.0	0.0	0.0	0.0	0.0	39.5
35	18.7	17.5	21.6	10.5	13.5	0.0	0.0	0.0	0.0	0.0	0.0	18.1

Note: Percent of total number of *L. salmonis* observed on each body zone of the experimental fish at each time point. See Fig. 1 for the different body zones. The developmental stages of lice at each sampling point are shown are Table 1.

reached the mobile stages (17 dpi). Detailed postmortem analysis was not undertaken, but most mortalities apparently were attributable to the effects of sea lice infestation.

Modelling

A three-way interaction between sea lice intensity, days after infestation, and the infested SW–FW group was retained for the AIC-determined model for plasma chloride. Because of the difficulties of interpreting a high-order interaction, the Bayesian information criterion (BIC; which incurs a larger penalty for each parameter) was used for comparison. In this case, a three-way interaction and two of its three associated two-way interactions were replaced by an interaction between the infested SW group and time. Because the reduction in predictive power for the BIC model was only marginal (0.503 vs. 475) and the prediction plots were virtually identical, the BIC model was chosen in the interests of parsimony. No significant differences were observed between groups at 0, 7, or 14 dpi for plasma chloride concentration (Fig. 2*a*). After 19 dpi, when the sea lice had reached the mobile stage (Table 1), plasma chloride in

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Table 4. Predictions of the mean intensity of *Lepeophtheirus salmonis* for the two infested groups

Group	Days after infestation	Mean *L. salmonis* intensity
SW infested	7	51.55
	14	100.43
	21	76.89
	28	64.93
	35	46.53
SW–FW infested*	7	70.52
	14	121.71
	21	62.97
	28	11.09
	35	1.65

*Fish in the seawater (SW) – freshwater (FW) transfer groups were transferred to FW at 19 days after infestation.

the infested SW group was significantly elevated above all over groups (21–35 dpi), whereas no significant difference was observed between the infested SW–FW and control groups.

A similar pattern of significance to that observed for plasma chloride was found for plasma lactate (Fig. 2*b*). At 14 dpi, however, plasma lactate was significantly elevated above control values in the infested SW–FW group, and at 21 dpi, it was significantly higher in both infested groups when compared with the controls. Plasma lactate in the infested SW group at 28 and 35 dpi was significantly elevated above both the control SW group and the infested SW–FW group (21 dpi), whereas no significant difference was observed between the infested SW–FW and control groups. There were no significant differences in plasma glucose concentration between groups at any time point; although there was some variation over time, the highest values in all groups were recorded at 21 dpi (Table 5) when almost all sea lice were at the mobile stages.

Liver glycogen concentration was extremely low at 0 dpi in all groups but had increased significantly for all groups by 7 dpi. There were no significant differences between any groups at 0 or 7 dpi. At 21 and 28 dpi, when the sea lice were mobile, liver glycogen was significantly lower in both infested groups compared with their respective controls. However, at 28 dpi, liver glycogen in the infested SW group was significantly lower than in the infested SW–FW group. At 35 dpi, a significant difference between the control SW and infested SW groups remained, but there was no significant difference between the control SW–FW and infested SW–FW groups at this time point.

Given the importance of plasma cortisol as a stress indicator, this marker was examined over time in further detail. Specifically, a separate model with potentially different covariates was fitted for each time point. No significant differences were observed for plasma cortisol until 14 dpi, when both infested groups were significantly elevated above their respective controls. At 21 dpi, plasma cortisol in the infested SW group was significantly elevated above the mean for all other groups. Data at 28 and 35 dpi were pooled owing to small sample sizes resulting from mortalities (seven fish were sampled from the infested SW group at 28 dpi and just six were from this group at 35 dpi). At 28–35 dpi, mean cortisol concentration was significantly higher in the infested SW group than the mean for all other groups.

Discussion

Sea lice development time and frequency distribution across the body surface were similar to those previously described for laboratory infestation of hatchery-reared Norwegian sea trout (Bjørn and Finstad 1998). In the present study, however, only a small proportion of copepodid and chalimus larvae were recorded from the gills in contrast to the 16% and 53% reported by Bjørn and Finstad (1998) and Johnson and Albright (1991*a*), respectively. No adult or preadult sea lice were found on the gills. The reason for the present discrepancy in mean sea lice infestation intensity between the four infested tanks remains unclear, but such variability is inherent to this type of experiment. All fish received infestation intensities considerably higher than the stress threshold intensity of 13 *L. salmonis*·fish^{-1} previously determined for Scottish sea trout smolts (mean weight 37 g) (Wells et al. 2006), and there were a number of sea lice induced mortalities in the infested SW group. As expected, by 28 and 35 dpi, the mean infestation intensity was significantly reduced in FW, confirming that sea lice were unable to survive transfer to FW for periods of more than a few days (Johnson and Albright 1991*a*). The inability of *L. salmonis* to survive FW transfer suggests that premature return to FW may be of benefit to sea trout postsmolts. This is supported by the markedly reduced mortality of infested fish returned to FW compared with the control SW group. It should be noted, however, that the experimental design did not allow assessment of the consequences of premature return over longer time periods. In particular, secondary bacterial or fungal infection was noted on a number of previously infested fish following their return to FW. Although these infections were not severe and did not appear to have major physiological consequences over the time period examined, it is possible that over a more prolonged period such FW infections may prove deleterious to the fish.

Mean plasma chloride levels remained stable throughout in the control SW group. Significant differences between the control and infested groups were observed only after *L. salmonis* had reached the mobile stages (21 dpi), in agreement with a previous study of wild sea trout smolts in Scotland (Wells et al. 2006). Plasma chloride continued to increase with time for the infested SW group, but the infested SW–FW group showed no significant difference from control values. Increases in plasma chloride for infested fish in SW may have resulted from skin damage associated with the increased grazing activity of late chalimus and mobile preadult and adult lice, in addition to stressor effects on osmoregulation (S.E. Wendelaar Bonga and I. Russon, unpublished data). Disturbance of water balance and ion homeostasis is one of the most characteristic aspects of stress in fish, primarily owing to the intimate relationship between the body fluids in the gills and the ambient water (Wendelaar Bonga 1997). The disturbance of hydromineral balance may arise as a result of marked structural changes in the gills (S.E. Wendelaar Bonga and I. Russon, unpublished data), which themselves are characteristic of stress. Return to FW and the associated loss of *L. salmonis* therefore alleviated the

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Fig. 2. Predicted mean values with 95% confidence intervals for (*a*) plasma chloride, (*b*) plasma lactate, (*c*) liver glycogen, and (*d*) plasma cortisol: control seawater (SW), solid line and open circle; control seawater–freshwater (SW–FW), broken line and solid circle; infested SW, broken line and open triangle; infested SW–FW, dotted line and solid triangle. The vertical lines represent the point at which two groups were returned to FW (19 days after infestation). Data are slightly offset for clarity.

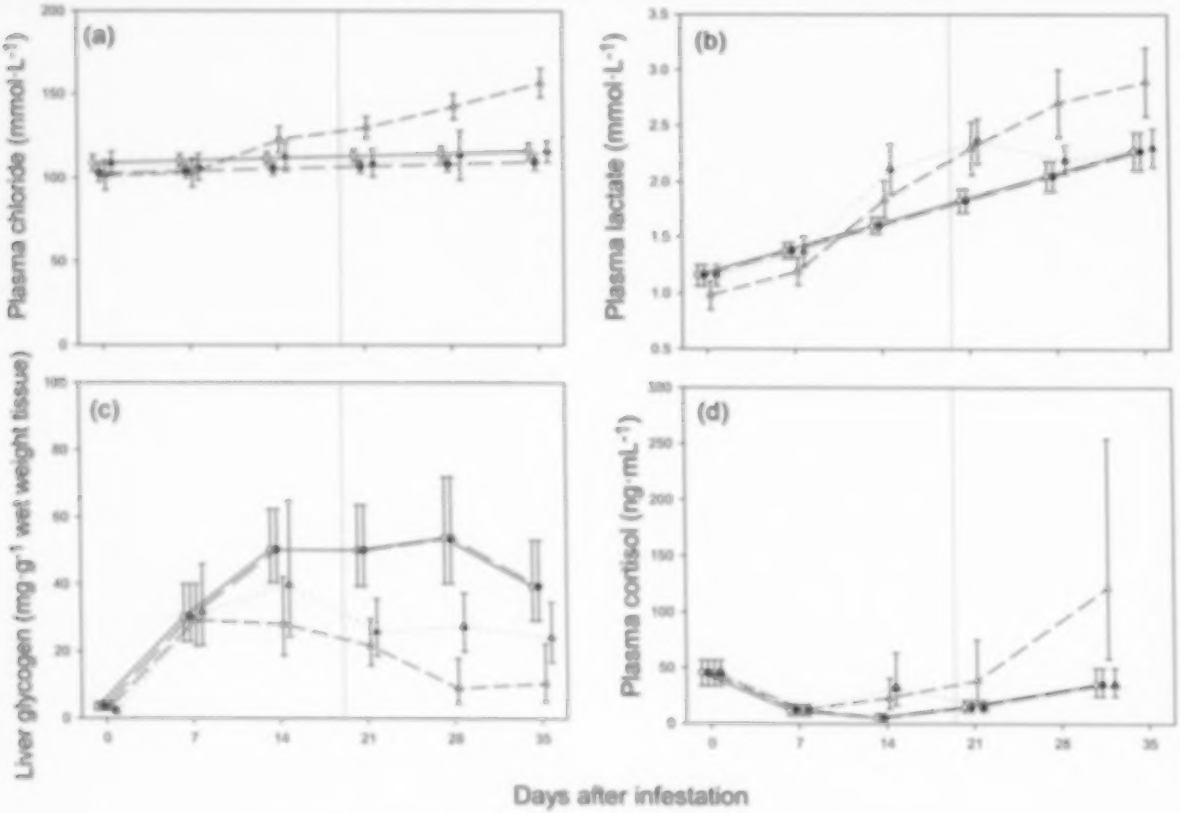

Table 5. Predicted mean values (mmol·L^{-1}) with 95% confidence intervals for plasma glucose concentration.

Days after infestation	Control SW	Infested SW	Control SW–FW*	Infested SW–FW*
0	3.50 ±0.33	3.50 ±0.33	3.50 ±0.33	3.48 ±0.43
7	3.92 ±0.42	3.50 ±0.35	3.92 ±0.42	4.10 ±0.48
14	5.69 ±0.50	4.50 ±0.70	5.69 ±0.50	6.04 ±1.03
21	6.58 ±0.63	6.40 ±0.73	6.58 ±0.63	7.66 ±0.87
28	5.20 ±0.57	3.39 ±1.01	5.20 ±0.57	4.95 ±0.60
35	4.31 ±0.48	3.87 ±1.06	4.31 ±0.48	4.29 ±0.56

*Fish in the seawater (SW) – freshwater (FW) transfer groups were transferred to FW at 19 days after infestation.

marked osmoregulatory disturbances that persisted for infested fish in SW. Premature return may therefore be of general physiological benefit to compromised postsmolt sea trout.

Hyperglycemia (elevation of plasma glucose) and elevated plasma lactate levels have been reported during both acute and chronic stress and are seen as useful stress indicators in fish (Farbridge and Leatherland 1992; Iversen et al. 1998), but the responses are variable and species-specific (Barton 2000). Stressor-induced anaerobic conditions can result in the breakdown of muscle glycogen to lactate, with some of this lactate being released into the bloodstream (Wood 1991). However, it is important to note that liver glycogen, plasma glucose, and plasma lactate all are also influenced by the overall metabolic status of the animal. In the present study, the experimental migrant smolts were intercepted following overwintering and several months of fasting at low temperature. Experimental feeding to satiation following natural protracted fasting may have led to increases in meta-

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bolic markers, independent of any sea lice stress effects. In rainbow trout (*Oncorhynchus mykiss*), plasma glucose and liver glycogen increased rapidly after the resumption of feeding in starved fish in the absence of any increases in plasma cortisol (Pottinger et al. 2003). In this context, it is notable that plasma glucose was elevated in all groups by 21 dpi compared with at 0 dpi.

Plasma lactate increased in the infested SW group and was elevated above the controls by 21 dpi and above all other groups by 28 dpi. Infested fish showed a variable degree of distinct "flashing" behaviour or jumped clear of the water surface, particularly during the infestation procedure and subsequently when the lice reached the mobile stages. Such behaviour, which has been described previously in response to sea lice infestation (Wootten et al. 1982), may have contributed to the observed increased plasma lactate levels. However, this behaviour was not quantified in the current study. Plasma lactate was also elevated above control values at 14 and 21 dpi in the infested SW–FW group but did return to control values by 28 dpi. The return of plasma lactate concentration to control values following return to FW may indicate a specific benefit of premature return to FW for infested fish.

The winter period of fasting immediately before the experiment was reflected in the initial extremely low liver glycogen levels (2.2–3.4 $mg \cdot g^{-1}$ tissue wet weight). Liver glycogen increased rapidly in all groups until 14 dpi, probably as a function of the fish being fed to satiation. At 21 dpi, once the lice had reached the mobile preadult stages, liver glycogen was significantly higher in the two control groups than in their respective infested groups. Liver glycogen remained depressed in the infested SW group, but following return to FW, there was evidence of some recovery in the infested SW–FW group. At 35 dpi, there was no significant difference in liver glycogen content between the infested SW–FW group and the respective control group.

Reductions in liver glycogen in infested fish may reflect an increased energy demand to the host animal, perhaps due to increased metabolic requirements such as the maintenance of osmoregulation following physical damage. Cortisol has been shown to suppress appetite in teleost fish (Mommsen et al. 1999). Throughout the present experiment, fish were fed to satiation, but there was reduced consumption by infested fish once sea lice attained the mobile stages. Individual consumption was not quantified, but it is possible that the decrease in liver glycogen was not a direct result of cortisol-induced stress, but rather an indirect consequence of reduced feeding. Fish that were returned to FW had the same access to food as previously in SW, and liver glycogen content did recover to some extent for infested fish in FW. The likelihood is, however, that wild, prematurely returning sea trout postsmolts would encounter reduced food availability in FW, perhaps to the extent of compromising liver glycogen stores yet further.

An elevation in plasma cortisol is the most widely used indicator of stress in fish (Wendelaar Bonga 1997). Primary stress, such as release of catecholamines and cortisol, may cause structural changes in the gills, for example, through epithelial lifting (fluid infiltration in the intercellular spaces and separation of the epithelia from its basal membrane; Wendelaar Bonga and Balm 1999). These changes will have pronounced effects on the major functions of the gill, often leading to disturbance of water and ion homeostasis (Mallatt 1985). Cortisol also stimulates controlled cell death (apoptosis) of the branchial epithelial cells and the promotion of differentiation of new chloride cells (Wendelaar Bonga 1997). In the present study, plasma cortisol increased significantly in both infested groups; this was apparent at 14 dpi, in contrast to our previous study on wild sea trout postsmolts in which significant increases were observed only once *L. salmonis* had moulted to the mobile stages (Wells et al. 2006). By 28–35 dpi, only the infested SW group had a plasma cortisol concentration significantly above control levels. This elevation of plasma cortisol is consistent with the well-characterised stress response (Wendelaar Bonga 1997), and the lack of a chronic increase in plasma cortisol in the infested SW–FW group again indicates that premature return to FW is beneficial to sea lice infested postsmolt sea trout.

This is the first empirical study of the physiological effects of premature return on sea trout postsmolts, using a series of osmoregulatory, metabolic, and stress measures and GAM modelling. Return to FW resulted in the expected loss of *L. salmonis* from the body surface of infested sea trout smolts within 1 week, and mortality in the infested SW–FW group was considerably lower than in infested fish maintained in SW throughout. Significant increases in plasma chloride, lactate, and cortisol and a significant decrease in liver glycogen content were apparent for infested fish held in SW, but all of these physiological effects were alleviated in fish that were returned to FW. Premature return of sea lice infested postsmolt sea trout does, therefore, confer considerable osmoregulatory and metabolic benefits to the host fish. However, a balanced judgement must be drawn between short-term beneficial effects of premature return versus the reduced feeding opportunities for wild fish in FW and possible secondary infection of damaged epithelia. These latter effects might have long-term consequences for the survivorship and future fecundity of sea trout populations heavily impacted by sea lice in the marine environment.

Acknowledgements

This study was carried out with the support of European Commission DG Fisheries Contract No. Q5RS-2002-00730 "Sustainable Management of Interactions Between Aquaculture and Wild Salmonid Fish (SUMBAWS)".

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Influence of velocity and chlorophyll standing stock on periphyton $\delta^{13}C$ and $\delta^{15}N$ in the Ste. Marguerite River system, Quebec

Joseph B. Rasmussen and Veronique Trudeau

Abstract: In a previous laboratory experiment, water velocity had a strong negative effect on periphyton $\delta^{13}C$ and $\delta^{15}N$, probably reflecting the effect of flow on boundary-layer exchange. To determine whether similar patterns could be detected in situ at different spatial scales, we examined several reaches, each with a wide range in water velocity and chlorophyll standing stock. Periphyton samples were uniform in composition, composed predominantly of living and dead diatoms of very few species. $\delta^{13}C$ signatures were more variable (–17.7‰ to –31.5‰) than $\delta^{15}N$ signatures (–0.4‰ to 5.7‰). Velocity and chlorophyll were significant predictors of periphyton $\delta^{13}C$ within reaches, mainly at medium scales (10–500 m) where persistent alternations between fast and slow current (riffles–runs–pools) produced inverse fluctuations of periphyton $\delta^{13}C$. However, none of the variability in $\delta^{15}N$ could be explained, possibly a result of severe boundary-layer N depletion. Similarly, the velocity and chlorophyll predictors could not explain the among-reach signature differences for either isotope. $\delta^{13}C$ signatures tended to be ^{13}C-depleted in the tributary streams and ^{13}C-enriched in the downstream reaches.

Résumé : Dans une expérience antérieure en laboratoire, la vitesse du courant avait un fort effet négatif sur le $\delta^{13}C$ et le $\delta^{15}N$ du périphyton, ce qui reflète probablement l'effet du courant sur les échanges dans la couche limite. Afin de déterminer si de tels patrons s'observent in situ à diverses échelles spatiales, nous avons examiné plusieurs sections présentant chacune une gamme étendue de courants et de biomasses de chlorophylle. Les échantillons de périphyton ont une composition uniforme et comprennent principalement un très petit nombre d'espèces de diatomées vivantes et mortes. Les signatures de $\delta^{13}C$ sont plus variables (–17,7 ‰ à –31,5 ‰) que celles de $\delta^{15}N$ (–0,4 ‰ à 5,7 ‰). La vitesse du courant et la chlorophylle sont des variables explicatives significatives du $\delta^{13}C$ du périphyton dans les sections, particulièrement aux échelles intermédiaires (10–500 m) où des alternances persistantes entre les zones de courants rapides et lents (rapides–coulées–fosses) causent des fluctuations inverses du $\delta^{13}C$ du périphyton. Toutefois, rien de la variabilité de $\delta^{15}N$ ne peut être expliqué, probablement à cause d'un important épuisement de N dans la couche limite. De même, les variables explicatives, la vitesse du courant et la chlorophylle, ne réussissent pas à élucider les différences dans les signatures de ni l'un ni l'autre des deux isotopes. Les signatures de $\delta^{13}C$ ont tendance à être réduites en ^{13}C dans les tributaires et enrichies en ^{13}C dans les sections d'aval.

[Traduit par la Rédaction]

Introduction

In recent years, stable isotopic signatures have become an essential ecological tool due to both their ability to reflect trophic dynamics and their importance as source indicators to provide spatial information about physical and biological processes. Interpreting stable isotopic signatures in food web studies requires understanding of the factors that contribute to spatial variability in primary producer signatures, including both the variability in fractionation relative to primary nutrient sources and the biogeochemical factors that influence nutrient signatures. In river systems, there is a complex interplay of these two factors operating at different spatial scales that makes consumer signatures even more challenging to interpret (Finlay et al. 1999, 2002; Finlay 2004). In a laboratory experiment, Trudeau and Rasmussen (2003) have shown that both ^{13}C and ^{15}N fractionation by benthic algae increase with water velocity, although the effect on $\delta^{13}C$ was much stronger. Finlay et al. (2002) found very similar results in a field study where they compared $\delta^{13}C$ and $\delta^{15}N$ signatures of benthic algae taken from riffles vs. pools. Reduced fractionation under low velocity likely results from nutrient depletion in a stagnant boundary layer, and high velocities likely prevent this from occurring. Moreover, thin diatom films had more negative $\delta^{13}C$ signatures than more dense communities dominated by filamentous green algae under the same velocity conditions, indicating that the thickness of the algal mat

Received 31 July 2006. Accepted 4 June 2007. Published on the NRC Research Press Web site at cjfas.nrc.ca on 7 October 2007. J19454

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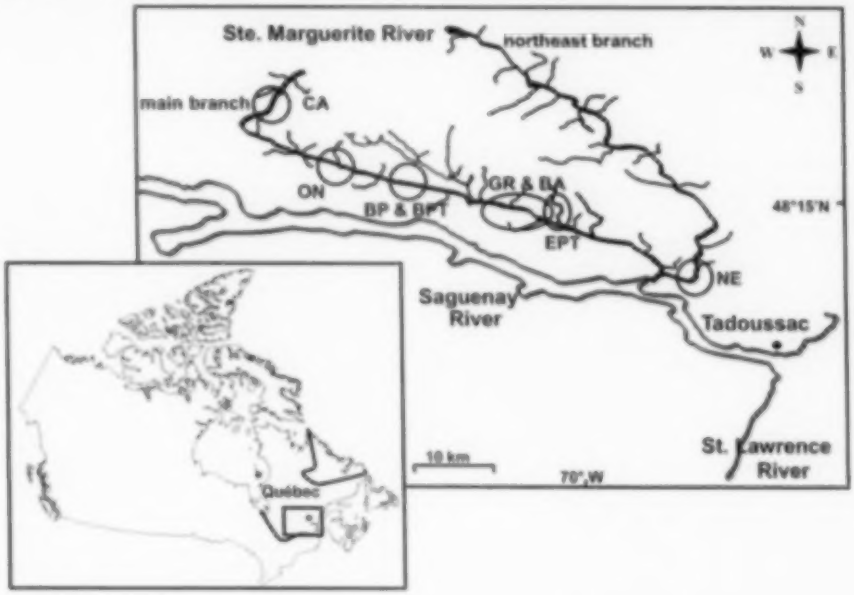
 doi:10.1139/F07-109 © 2007 NRC Canada

Fig. 1. The Ste. Marguerite River, Quebec, with the tributaries and reaches sampled during this study: EPT, Epinette tributary; BPT, Big Pool tributary; CA, Cascade; ON, Onesime; BP, Big Pool; GR, Grande Rapide; BA, Bardsville; NE, northeast reach.



(chlorophyll standing stock) might interact with water velocity to influence signatures, both through its physical presence in the boundary layer and by enhancing the uptake rate of nutrients by the biofilm. Recent studies have provided strong support for both the velocity and the chlorophyll effect. Singer et al. (2005) showed that under highly fluctuating velocities, periphyton $\delta^{13}C$ signatures strongly reflected flow histories over several weeks, and Hill and Middleton (2006) have shown that the buildup of chlorophyll standing stock over time on artificial substrates enriched periphyton $\delta^{13}C$ signatures. Signature effects resulting from boundary-layer effects on fractionation can also be traced up the food web. Finlay et al. (1999, 2002) were able to discriminate $\delta^{13}C$ signatures of fish and invertebrates from pools and riffles and used these to trace food web interactions. Morinville and Rasmussen (2003, 2006*a*) found that premigratory juvenile brook trout, which occupy faster water habitats than residents, also had more depleted carbon signatures than residents.

Although our controlled laboratory study generated patterns on a small spatial scale, where velocity was regulated and light and water source were kept constant, the present field study was conducted to examine in situ patterns of $\delta^{13}C$ and $\delta^{15}N$ in the Ste. Marguerite River (SMR), where many factors are varying simultaneously. Although flow conditions change gradually along the SMR from reach to reach, each section of the river has sections where flow alternates between fast (riffles) and slow (pools) velocity. By sampling periphyton communities along these gradients of velocity, as well as across the river, we hoped to generate a data set of periphyton $\delta^{13}C$ and $\delta^{15}N$ signatures that would allow us to model the relationships of signatures to velocity and chlorophyll standing stock across a range of spatial scales and determine how much of the spatial variation in isotopic signatures along the course of the river could be explained by these variables.

We expected that periphyton from river sections with persistent fast currents would have significantly more ^{13}C- and ^{15}N-depleted signatures than sections with persistent slow currents. In addition, we hypothesized that high chlorophyll standing stock should result in enriched signatures due to both enhanced nutrient flux and reduced boundary-layer exchange resulting from the thicker biofilm. Because the river exhibits velocity gradients through several different mechanisms that operate on different scales, we tested the hypothesis on (*i*) a local scale (<10 m), where cross-sectional gradients in velocity predominate, (*ii*) a medium scale (10–500 m), where alternating pool–run–riffle patterns occur, and finally, (*iii*) a larger scale (>500 m), where some reaches are steeper and have faster average current speeds than others.

Materials and methods

Study area

The study area was the Ste. Marguerite River system in the Saguenay region, Quebec, Canada (Fig. 1). This river, with its two major branches, drains a 1800 km^2 area (runoff coefficient 600 mm; Fisheries and Environment Canada 1978), receives 1140 mm of precipitation annually, 30% of which falls as snow, and delivers an average discharge of 30.3 $m^3 \cdot s^{-1}$ (Good et al. 2001). Peak discharges are generally associated with the spring melt and exceed 100 $m^3 \cdot s^{-1}$ during late April through May; minimum flows occur during July and August and are usually <20 $m^3 \cdot s^{-1}$ (Good et al. 2001). Water chemistry was monitored weekly for 11 weeks at 4–6 stations in four study reaches (i.e., Cascade, Onesime, Big Pool, Grande Rapide – Bardsville), during summer 2003 by J. Luce and M. Lapointe (Department of Geography, McGill University, Montréal, Qué-

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bec; Table 1). Like most rivers and streams on the Canadian Shield, especially those of the upper north shore of the Gulf of St. Lawrence, the Ste. Marguerite has very low conductivity, alkalinity, dissolved inorganic carbon (DIC) (Lachance et al. 1988), and nutrient concentrations, although its values for total N and P are above the median values for Shield streams given by Grenier et al. (2005).

Many streams feed the river and there are no polluting industrial sources within the drainage system or recent impacts of clear-cut logging operations. Because we had limited road access to much of the river, we performed detailed sampling in a total of eight reaches: five in the main branch of the river, one in the northeast branch, and one in each of two easily accessible tributaries. The term reach, as we apply it, has no definitive meaning in terms of hydrology or river geometry. By reach, we simply refer to a river or tributary section composed of a series of nearby sites encompassing a series of pool–run–riffle sequences. The names applied to the reaches we studied are traditional names used by salmon guides. The river reaches were up to a few kilometres in length, whereas tributary reaches were only a few 100 m in length. The five reaches in the main branch are listed in order from downstream to upstream in Fig. 1. Bardsville (BA) is a wide section (>40 m) with no canopy cover. Just upstream, Grande Rapide (GR) is similarly wide with no canopy cover but is steeper with many boulders. Further upstream, Big Pool (BP) and Onesime (ON) are 10 and 20 km, respectively, upstream of GR. These reaches are narrower (10–20 m), with small trees and shrubs along both banks. The upstream-most section sampled, Cascade (CA), was the narrowest (≈10 m), shallowest, and fastest reach, with well-treed banks and more canopy cover than any other reach on the main river. The stream reaches Epinette tributary (EPT) and Big Pool tributary (BPT) are very narrow (on average 4 and 2 m wide, respectively) and shallow with a continuously high percent canopy cover. Although a canopy cover is present, it should be noted that this is a boreal forest region with a history of logging and contains no tall coniferous or deciduous trees. Consequently, none of the streams is shaded to anywhere near the same extent as first- and second-order streams in beech maple forests further south. Moreover, periphyton growth is always abundant in these streams. The NE reach is situated in the northeastern branch of the river below the falls. This reach is typical of the lower section of the river, which has no canopy cover, a gentle slope, and silty gravel. Some periphyton samples were also taken from the lower river (LR), near its mouth. This section was not sampled consistently, as it was not possible to locate sites with a wide spectrum of flow velocities. However, a few samples were taken in this rich silty clay section for comparison with results from the NE reach.

Species collected

Periphyton samples were examined microscopically and found to be very uniform and composed mainly of living and dead diatoms, mainly *Tabellaria* spp., *Eunotia* spp., *Navicula* spp., *Synedra* spp., and *Gomphonema* spp. At the time of sampling, almost no green filamentous algae (Chlorophyta) was found in the river and associated streams. Some species were found in low proportion in a few samples (*Tetrasporu* spp., *Bulbochaete* spp., *Draparnaldia* spp., and *Ulothrix* spp.). A few species of cyanobacteria also accounted for a small propor-

Table 1. Water chemistry and nutrient concentrations in four study reaches of the Ste. Marguerite River.

Reach	Total N, μmol N·L^{-1}	NH_4^+, μmol N·L^{-1}	NO_3^-+NO_2^-, μmol N·L^{-1}	Total P, μmol P·L^{-1}	PO_4^{-3}-P, μmol P·L^{-1}	Silica, μmol Si·L^{-1}	HCO_3^-, μequiv·L^{-1}	DIC, μmol·L^{-1}	pH	Cond., μS·cm^{-1}	*N*
Cascade	20.7±1.5	1.48±0.16	2.87±0.39	0.87±0.18	0.42±0.03	91.2±13.4	—	—	—	—	4
Onesime	17.1±1.96	1.84±0.78	2.78±0.39	1.48±0.36	0.53±0.19	96.1±1.33	—	—	—	—	4
Big Pool	16.1±1.21	1.18±0.19	3.43±0.49	1.34±0.20	0.38±0.20	112±12.4	—	—	—	—	4
Grande Rapide – Bardsville	14.3±0.75	1.04±0.17	3.48±0.29	0.92±0.15	0.40±0.04	91.2±3.4	—	—	—	—	6
Ste. Marguerite mean	17.1	1.40	3.14	1.15	0.43	—	120[a]	130[a]	7.2	26	18
Canadian Shield mean[b]	15	1.43	3.71	0.55	0.32	—	—	—	7.3	38	—

Note: Unpublished data provided by James Lace and Michelle Lapointe, Department of Geography, McGill University, Montréal, Québec (represents sampling over 11 weeks during June–August 2003). DIC, dissolved inorganic carbon; Cond., conductivity; *N*, number.

[a]After Lachance et al. (1988). DIC calculated as HCO_3^- alkalinity + $K_H pCO_2$ (= 10 μequiv·L^{-1}, assuming pCO_2 = 3.7 × 10–4 atm) following Stumm and Morgan (1996)).

[b]After Grenier et al. 2005.

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tion of the communities (*Gloeotrichia* spp. and *Phormidium* spp.). Although some fine particulate detritus (mostly leaf fragments) and conifer pollen grains were often present in periphyton samples, and these were not removed, their contribution to mass would have been negligible, and there was little doubt that the measured periphyton signature did not include a significant proportion of terrigenous material. Although it is possible that some of the dissolved organic matter being used by bacteria within the periphyton community may be of terrestrial origin, their biomass is still being formed in situ and can be considered autochthonous in that sense.

Water velocity measurements

In every reach, 7 to 15 sites (cross sections) were chosen according to their overall water velocity: pool (≤0.1 $m \cdot s^{-1}$), run (0.1–0.3 $m \cdot s^{-1}$), or riffle (>0.3 $m \cdot s^{-1}$). Consecutive sites within a reach were always different in terms of overall water velocity. Reaches varied in length and were generally ≈300 m in the tributary streams, where sites were closer together, and up ≈2 km in the river. Our sampling locations did not exceed 70 cm in depth, and rock sizes varied from 5 to 30 cm in diameter. Within each site, three different water velocities were measured using a Gurley pygmy current meter (625DF8N, wading rod suspended pygmy-type current meter outfit with model 1100 digital indicator; Gurley Precision Instruments, Troy, N.Y.). Water velocity measurements were taken 1.5 cm above the surface of the periphyton layer and in the middle of a 0.1 m^2 surface area delimited by a quadrat of the same size. The rocks exposed to the surface within the sampling quadrat were removed, and periphyton were completely washed off the rocks with a brush and a known volume of water. A subsample of this water–algae mixture was filtered using Whatman 47 mm glass microfibre filters and kept in the dark for subsequent chlorophyll *a* analysis. Another subsample was kept for stable isotopic analysis. All samples were frozen at –20 °C until analysed.

$\delta^{13}C$ and $\delta^{15}N$ have been reported to vary seasonally (Doi et al. 2003; Finlay 2004). Although preliminary analyses had failed to detect differences between early- and late-summer isotopic signatures, to avoid possible biases due to small temporal isotopic variations and changes in the water level, all reaches were sampled over a 3-week period during midsummer (July), when productivity is the highest. Sampling of individual reaches took no more than 2–3 consecutive days during which the water level stayed fairly constant (approximately ±2 cm).

Sample analyses

Stable isotope analyses

All samples of periphyton were oven-dried, pulverised, and placed into tin capsules. Stable C and N isotopic analyses were performed by mass spectrometry (Finnigan-Mat Delta-Plus continuous flow isotope-ratio mass spectrometer coupled to a Carlo-Erba elemental analyser online; G.G. Hatch Isotope Laboratories, University of Ottawa, Ottawa, Ontario, Canada, and GEOTOP Laboratory, University of Quebec in Montreal, Quebec, Canada). The analytical precision of these apparatus is typically 1 standard deviation (SD) for C and N and is in the range of 0.05‰ to 0.2‰, which is small relative to the range of values found in nature (Kendall and Caldwell 1998). Stable isotopic signatures were expressed using the following standard equation:

$$\delta^{13}C \text{ and } \delta^{15}N = [(R_{sample} / R_{standard}) - 1] \times 1000$$

where R is the ratio of ^{13}C to ^{12}C or ^{15}N to ^{14}N, and the standards are Pee Dee Belemnite limestone and atmospheric nitrogen for C and N, respectively. The units are in parts per thousand (‰).

Chlorophyll a analysis

Chlorophyll *a* was extracted for 24 h from each periphyton sample using 95% acetone. Chlorophyll *a* concentrations (expressed in $mg \cdot m^{-2}$) were then measured by spectrophotometry (Parsons et al. 1984), because microalgal chlorophyll *a* concentration is a good indicator of microalgal biomass (Wetzel and Likens 2000).

Data analyses

Descriptive statistics (mean, standard deviation) were calculated for periphyton $\delta^{13}C$ and $\delta^{15}N$, water velocity, and chlorophyll standing stock for each of the eight study reaches. Within each reach, linear regressions were calculated, and the parameters were compared among reaches. Using analysis of covariance (ANCOVA; general linear model in JMP-IN®, SAS Institute Inc., Cary, North Carolina), we then analyzed the effect of velocity and chlorophyll standing stock on periphyton signatures at the local scale (<10 m), at the intermediate averaging scale within reaches (10–500 m), and at the large scale encompassing entire reaches (>5 km).

Allocating total and explained variance to different scale intervals

Because gradients in velocity can exist both across the river and along the river at different spatial scales, we attempted to partition total variance and variance explained by the test variables velocity and chlorophyll standing stock into increments reflecting different spatial scales. These analyses of scale effects were carried out on the main branch, where sampling was most intensive, and involved two different procedures. (*i*) The first was data smoothing, which combined individual periphyton samples (0.1 m^2), and the velocity and chlorophyll measurements taken with them, into groups of samples located within 10 m (site means) and 500 m of each other along the river, and calculating the average for each variable for each of these groupings. In a few cases, sites belonged to more than one grouping, but no groupings that shared more than half of their sites, were counted in these analyses. Variances among each of these groupings were then calculated for each variable, and the explanatory power of the predictors and the categorical REACH effect in each ANCOVA analyses were determined for all of the grouped data sets. (*ii*) In the second, variables were standardized with respect to specific scale means by subtracting the scaled group means from each data point. By combining the smoothing and the standardization procedures, increments of total and explained variance were estimated for three intervals of spatial scale: (1) local scale (<10 m) that mostly reflect cross-sectional variation at a point in the river), (2) medium scale (10–500 m), the scale at which river meanders produce large velocity gradients associated with alternating pool–run–riffle sequences, and

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(3) large scale (>500 m), where variations in bed slope, channel width, and depth can give rise to velocity variations. For the analysis of local-scale effects, data from each individual sampling location were standardized to site averages by subtracting the site mean from each data point at that site. For the analysis of medium-scale effects, site average values were standardized to 500 m group averages. Finally, for the analysis of large-scale effects, 500 m group averages (unstandardized) were used for each variable.

Results

Water velocities ranged from 0 to 1.27 $m \cdot s^{-1}$, which spans the range normally encountered in rivers and streams (Table 2). Although the range was large in all reaches, mean velocities differed among reaches, with the fastest currents recorded in the steep, narrow upper reaches of the main branch (CA and ON), and slower currents recorded in the wider, less steep sections downstream. The lowest mean velocities were found in the small tributary EPT. Chlorophyll standing stock ranged from 0.3 to 31.1 $mg \cdot m^{-2}$, and both variables were highly variable within each reach (Table 2). The highest mean chlorophyll was in the NE branch, and the lowest means were in the steep upstream sections of the main branch. Periphyton $\delta^{13}C$ varied from –31.5‰ to –17.7‰ over all the reaches, and within-reach variability was also high, with ranges between 3.6‰ and 7.8‰. All reaches in the main branch, as well as the BPT reach, had similar mean $\delta^{13}C$ between –25.3‰ and –26.7‰. The EPT reach had the most negative $\delta^{13}C$ mean (–29.7‰) and the smallest range, whereas the NE reach had the least negative $\delta^{13}C$ mean (–21.0‰) and the largest range. The mean $\delta^{15}N$ of individual reaches were very similar and varied from 1.1‰ to 3.1‰, with the highest values recorded in the lower section of the river (NE and LR).

Periphyton $\delta^{13}C$ and $\delta^{15}N$ versus velocity and chlorophyll standing stock

Within reaches, water velocity and periphyton $\delta^{13}C$ (site means) tended to oscillate inversely along the pool–run–riffle sequences (Fig. 2); thus when water velocity was high, $\delta^{13}C$ was low and vice versa. Within each reach, the $\delta^{13}C$ versus velocity regressions were highly significant ($p < 0.002$) (Fig. 3). The intercept values differed among reaches, ranging from –29.1‰ in EPT to –19.8‰ in the NE branch. The intercepts tended to vary in the same direction as the reach means. The reach-specific $\delta^{13}C$ versus chlorophyll regressions ranged from highly significant ($p < 0.001$) in ON, BP, and the NE branch to nonsignificant in EPT and GR (Fig. 4). The most significant of these relationships were curvilinear, requiring a quadratic fit with negative second-power term. Because chlorophyll standing stock tended to decline slightly with increasing velocity (Fig. 5), the interaction term log(chlorophyll $a \cdot$ velocity^{-1}) also yielded highly significant regressions in each reach (Table 3). For most reaches, multiple regressions containing velocity and chlorophyll a terms were significant. The multiple regressions were not significantly improved by power or logarithmic transformation of the variables, or by the inclusion of

Table 2. Descriptive statistics of periphyton $\delta^{13}C$ and $\delta^{15}N$, velocity, and periphyton biomass (Chl a) along the Ste. Marguerite River System for each study reach.

		Tributaries		Main branch (middle section from upstream to downstream)					Main branch (lower section)	NE branch (lower section)
		EPT	BPT	CA	ON	BP	GR	BA	LR	NE
$\delta^{13}C$ (‰)	Mean	–29.75	–26.68	–25.25	–26.86	–26.67	–26.05	–26.13	–20.05	–21.16
	(±SD)	(±0.94)	(±1.19)	(±1.35)	(±1.78)	(±1.27)	(±1.53)	(±2.13)	(±0.40)	(±2.35)
	N	44	39	44	23	27	19	33	3	35
$\delta^{15}N$ (‰)	Mean	2.05	1.11	—	2.89	2.41	2.03	1.80	5.0	3.14
	(±SD)	(±1.32)	(±0.55)		(±0.88)	(±0.76)	(±0.73)	(±0.86)	(±1.10)	(±0.97)
	N	45	40	—	23	21	12	26	3	39
Velocity ($m \cdot s^{-1}$)	Mean	0.21	0.37	0.49	0.51	0.36	0.22	0.32	0.10	0.30
	(±SD)	(±0.19)	(±0.30)	(±0.29)	(±0.23)	(±0.21)	(±0.17)	(±0.22)	(±0.03)	(±0.29)
	N	45	42	45	24	9	21	33	3	39
Chl a ($mg \cdot m^{-2}$)	Mean	8.06	7.24	5.34	5.21	4.63	7.27	7.48	8.9	12.2
	(±SD)	(±2.46)	(±5.35)	(±3.46)	(±3.95)	(±5.17)	(±5.26)	(±3.90)	(±3.50)	(±7.08)
	N	45	42	41	24	27	21	33	3	38

Note: $\delta^{15}N$ measurements for the CA reach were lost as a result of a machine malfunction. EPT, Epinette tributary; BPT, Big Pool tributary; CA, Cascade; On, Onesime; BP, Big Pool; GR, Grande Rapide; BA, Bardsville; SD, standard deviation; Chl a, chlorophyll a.

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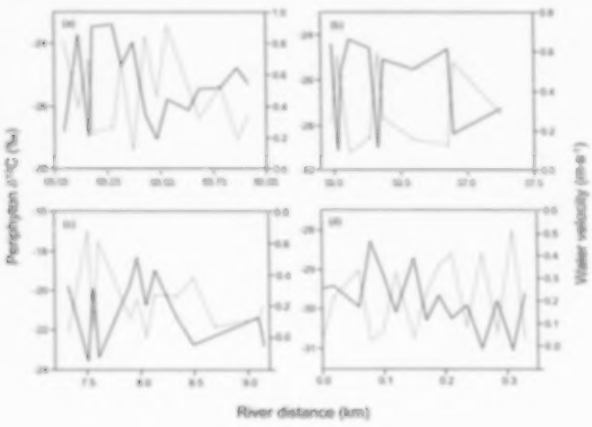
Fig. 2. Along-stream pattern of site mean Periphyton $\delta^{13}C$ (solid line) and site mean water velocity (dotted line) vs. river distance for four representative reaches. For (*a*) Cascade (CA), (*b*) Bardsville (BA), and (*c*) northeast (NE) reaches, distance (km) was measured relative to the mouth of the river where the Ste. Marguerite River enters Baie Ste. Marguerite; for panel (*d*) the Epinette tributary reach (EPT), distance was measured upstream from an arbitrary point.



second-power (quadratic) terms for chlorophyll and interaction terms together with velocity and chlorophyll *a*.

Having verified that velocity was an excellent predictor of periphyton $\delta^{13}C$ within reaches and that chlorophyll was at least significant in most reaches, we tested these predictors together on the entire data set using an ANCOVA with REACH as the categorical variable (Table 4). The latter ranged from –4.29 to +4.04 above the mean response of –26.15 (BA) around which the ANCOVA is centered. Both predictors, velocity (*t* value = –10.7) and chlorophyll (*t* value = 9.8), were highly significant in the presence of the categorical variable REACH, which explained the baseline differences in signatures between reaches not explained by velocity and chlorophyll. This model explained 85% of the variance in periphyton $\delta^{13}C$ across the entire data set. Although the slopes of periphyton $\delta^{13}C$ vs. velocity and chlorophyll obtained in reach-specific regressions were variable, interaction terms (velocity × REACH and chlorophyll × REACH) were not statistically significant.

Although significant differences among reaches were present for periphyton $\delta^{15}N$, none of the variation either among or within reaches could be explained by water velocity or chlorophyll or any interaction between them. Of the seven reaches (note that CA samples for $\delta^{15}N$ were lost), only BPT showed a barely significant positive, rather than a negative, linear regression with velocity. Of the six nonsignificant relationships, half were positive (BA, GR, and ON) and half were negative (BP, EPT, and NE).

Distinguishing small-scale from medium-scale effects

Variance in periphyton $\delta^{13}C$ increased with spatial scale, but the greatest proportion explained (70%) was at the medium-scale increment (10–500 m) (Table 5). This reflects the response to the variability in velocity imposed by the pool–run–riffle sequences linked to the river meanders. At the local scale (<10 m), the explanatory power of the predictors was much weaker (12%). Although the total variance was much greater at the larger scale, it was not explainable by velocity and chlorophyll, indicating that this large-scale variance is most likely due to hydrological and (or) biogeochemical differences among reaches.

The spatial pattern in $\delta^{13}C$ observed at the medium scale and the associated changes in water velocity across pool–run–riffle sequences are illustrated in Fig. 2, where $\delta^{13}C$ (site means) are plotted alongside water velocity against distance for three representative main river reaches and a stream tributary. Each oscillation in water velocity is clearly linked to an inverse oscillation in $\delta^{13}C$. The sequence of alternation between fast and slow water takes place over scales of a few hundreds of metres in the main river reaches and a few tens of metres in the stream tributary, and averaging at larger scales largely smooths them out. Thus the scale at which the predictors explain the greatest proportion of variance in $\delta^{13}C$ (Table 5) corresponds to the spatial scale of the pool–run–riffle alternation sequence along the river.

Discussion

A number of factors have been proposed to influence periphyton $\delta^{13}C$ signatures, including water velocity (Finlay et al. 2002; Trudeau and Rasmussen 2003; Singer et al. 2005), chlorophyll standing stock (Hill and Middleton 2006), DIC concentrations and signatures (Finlay 2001, 2004), temperature (Hill and Middleton 2006), and light (MacLeod and Barton 1998). This study of spatial patterns in the SMR shows that water velocity and chlorophyll standing stock are both highly significant predictors of within-reach variability in periphyton $\delta^{13}C$, but that among-reach variation is more likely related to other factors that were not measured. The most plausible explanation for the among-reach differences is that the $\delta^{13}C$ signature of the DIC becomes gradually enriched downstream, as a result of a combination of factors including atmospheric signature equilibration, degassing of excess CO_2, and the effects of instream processes such as isotopic fractionation by periphyton (Finlay 2003).

This trend is similar to the upstream–downstream trend in periphyton $\delta^{13}C$ reported by other authors (Doucett et al. 1996; Finlay 2001). The effects of temperature and light were not examined during this study but may also have contributed to unexplained variability in our models. Although our study supported the importance of previously hypothesized factors, the analysis showed that their explanatory power depended greatly on the spatial scale of analysis.

Effects of velocity and chlorophyll on small- and medium-scale variation in $\delta^{13}C$

Analyses based on smoothing and standardization of variables indicated that the explanatory power of velocity and chlorophyll was very low at the local (cross-sectional) scale (12%), despite the considerable amount of variance present at that scale in both the dependent and independent variables. The explanatory power of the predictors was by far the highest at the medium scale (>70%), reflecting the important contribution from the alternating pool–run–riffle sequences associated with the river meander. The inability of

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Fig. 3. Periphyton $\delta^{13}C$ regressed against water velocity (Vel) for each study reach: (*a*) Epinette tributary (EPT), (*b*) Big Pool tributary (BPT), (*c*) Cascade (CA), (*d*) Onesime (ON), (*e*) Big Pool (BP), (*f*) Grande Rapide (GR), (*g*) Bardsville (BA), and (h) northeast (NE).

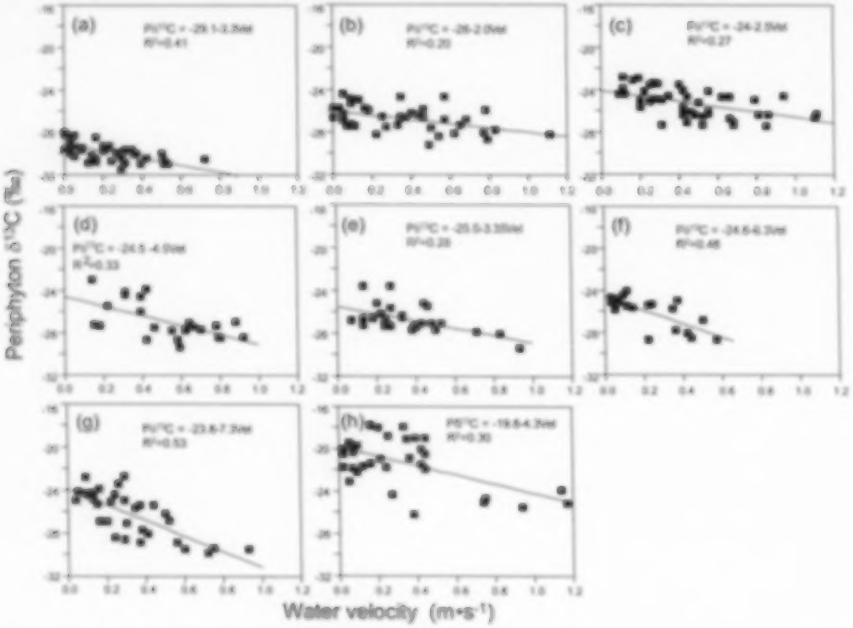
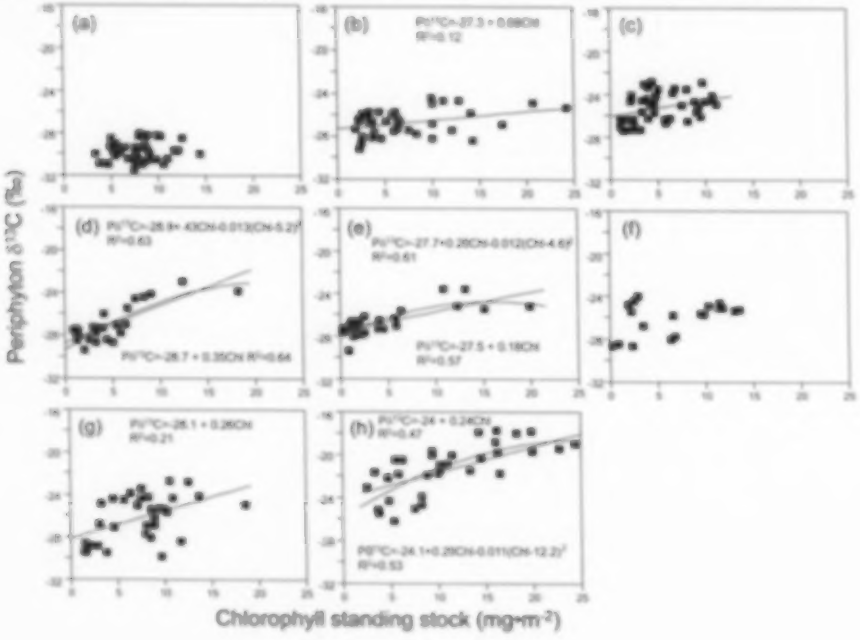


Fig. 4. Periphyton $\delta^{13}C$ vs. chlorophyll standing stock for each study reach: (*a*) Epinette tributary (EPT), (*b*) Big Pool tributary (BPT), (*c*) Cascade (CA), (*d*) Onesime (ON), (*e*) Big Pool (BP), (*f*) Grande Rapide (GR), (*g*) Bardsville (BA), and (h) northeast (NE). Statistically significant relationships are accompanied by a linear and (or) polynomial fitted equation and its R^2 value.



the predictors to explain periphyton $\delta^{13}C$ signature variation at the large scale was likely a result of the signature variation associated with the pool–run–riffle alternations having been smoothed out, leaving mainly among-reach variance related mostly to source signature and not velocity or chlorophyll.

The reason why the velocity gradients, formed along the river through pool–run–riffle sequences, correlate much better

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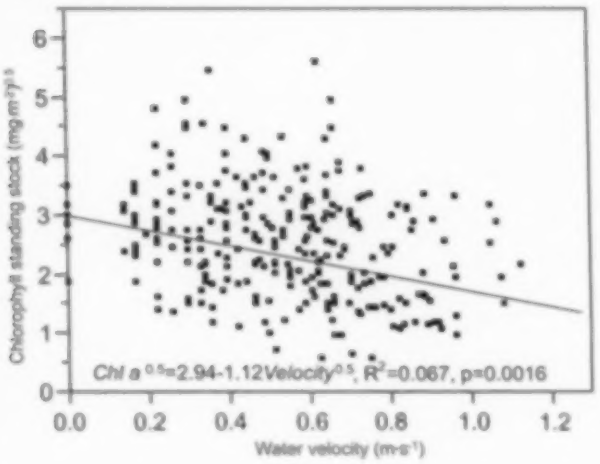
Table 3. Reach-specific regression models predicting periphyton $\delta^{13}C$ from velocity and chlorophyll *a* (Chl *a*) and their interaction term log(Chl *a*-velocity^{-1}) (dependent variable $\delta^{13}C$).

	EPT	BPT	CA	ON	BP	GR	BA	NE
Models predicting periphyton $\delta^{13}C$ from velocity and Chl *a*								
Intercept	−29.79	−25.95	−24.83	−27.02	−26.42	−24.60	−23.83	−22.56
Chl *a*	0.09	—	0.16	0.29	0.17	—	—	0.21
Velocity	−3.39	−2.00	−2.60	−2.65	−2.90	−6.29	−7.28	−3.39
Adjusted R^2	0.46	0.20	0.37	0.72	0.78	0.48	0.53	0.65
RMS	0.69	1.07	1.09	0.94	0.59	1.10	1.45	1.40
Models involving an interaction term between Chl *a* and velocity								
Intercept	−32.08	−28.00	−27.59	−29.64	−28.34	−28.34	−30.33	−26.00
Log(Chl *a*-velocity^{-1})	0.56	0.42	0.98	1.28	0.78	0.68	1.30	1.21
Adjusted R^2	0.45	0.22	0.46	0.61	0.65	0.49	0.54	0.44
RMS	0.64	1.07	1.01	1.10	0.74	1.10	1.45	1.77
N	44	39	41	23	27	19	33	34

Note: Only statistically significant terms were retained. EPT, Epinette tributary; BPT, Big Pool tributary; CA, Cascade; On, Onesime; BP, Big Pool; GR, Grande Rapide; BA, Bardsville; NE, northeast branch; RMS, root mean squared; *N*, number.

Fig. 5. Relationship between chlorophyll standing stock and water velocity across the whole data set.



with periphyton $\delta^{13}C$ than with local cross-sectional velocity gradients, which are often just as large, probably lies in the persistence of the gradients in time. Periphyton $\delta^{13}C$ signatures become gradually enriched over weeks to months as the standing stock builds up (Hill and Middleton 2006). Singer et al. (2005) have shown that under fluctuating velocity, flow history is a much stronger predictor of periphyton $\delta^{13}C$ signature than flows measured at a snapshot in time. This means that changes in the flow regime, caused by rises and falls in the water level during the growing season, may alter the water velocity at different points on the cross-sectional gradient to a much different degree. For example, samples taken at low water near the stream bank will be associated with much lower velocities than samples taken 10 m closer to midstream. However, if the water level rises by 50 cm, both sites would likely be far from the bank and their velocity regimes might be nearly the same. Thus, velocities measured only once may poorly reflect the flow history at any given site. On the other hand, sampling stations in a riffle should always be in a faster current regime than stations within a pool, even though both will experience increases and decreases in current speed as the river rises and falls. It is likely the consistency of the relative gradient that results in strong correlations between measured flows and signatures.

Large-scale patterns in periphyton $\delta^{13}C$

Steeper upstream reaches in the main branch with fastest mean current velocities and low mean chlorophyll (CA and ON) were expected to exhibit lighter periphyton $\delta^{13}C$ signatures than slower downstream reaches with higher chlorophyll standing stock, yet this was not observed. The only evidence that could support the effect of velocity on among-reach $\delta^{13}C$ differences was the strongly enriched signatures found in the lower portion of the NE branch, a reach with lower than average velocity and high chlorophyll levels. On this basis, the low velocity and high biomass in EPT should have produced heavy signatures, but instead, EPT periphyton had the most depleted C signatures of all the reaches. Because there were no significant relationships between the reach means of $\delta^{13}C$ and those of velocity, periphyton biomass, or their interaction terms, there is no statistical basis for among-reach differences being attributable to velocity or chlorophyll standing stock. However, only eight reaches were studied and it is possible that some significant effects may have been detected with greater statistical power.

Differences in mean periphyton $\delta^{13}C$ and intercepts between reaches most likely reflect reach-specific signature values of the DIC sources assimilated by the algae (Finlay 2004), and the most likely sources of these differences are atmospheric signature equilibration, degassing of excess CO_2, and the effects of in-stream processes such as isotopic fractionation by periphyton (Finlay 2003).

The EPT stream, with its mean of −29.7‰, had the most depleted periphyton $\delta^{13}C$, which is typical of small headwater streams and likely reflects the influence of soil weathering processes on the isotopic signatures of DIC (Boutton 1991; Finlay 2003). In the main branch, mean periphyton $\delta^{13}C$ estimates were fairly similar from one reach to another (−25.3‰ to −26.7‰). The signatures recorded in the BPT stream were not significantly more depleted than those of the five main branch reaches, probably because this reach was at a low elevation and not far upstream from its mouth. In the lower reaches of the

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Table 4. Analysis of covariance on the entire data set with the categorical variable REACH (dependent variable, periphyton $\delta^{13}C$ (‰)).

	Estimate	SE	t	Prob > |t|	
Intercept	−26.06	0.18	−143.70	<0.0001	
REACH (BA)	−0.23	0.19	−1.24	0.21	
REACH (BP)	−0.19	0.21	−0.91	0.36	
REACH (BPT)	−0.59	0.17	−3.44	0.00	
REACH (CA)	1.53	0.17	8.80	<0.0001	
REACH (EPT)	−4.29	0.17	−25.29	<0.0001	
REACH (GR)	−0.30	0.24	−1.27	0.21	
REACH (NE)	4.04	0.20	20.55	<0.0001	
Velocity ($m \cdot s^{-1}$)	−3.18	0.30	−10.74	<0.0001	
Chl *a* ($mg \cdot m^{-2}$)	0.16	0.02	9.76	<0.0001	
	Estimate	Source	F ratio	df	Prob > F
Adjusted R^2	0.84	REACH	17.48	7	<0.0001
RMS	1.13	Velocity	75.37	1	<0.0001
Mean of response	−26.15	Chl *a*	55.20	1	<0.0001
N	260				

Note: The estimates for the REACH effect are centered around the −26.06, which is the response for the BA reach. EPT, Epinette tributary; BPT, Big Pool tributary; CA, Cascade; On, Onesime; BP, Big Pool; GR, Grande Rapide; BA, Bardsville; SE, standard error; RMS, root mean squared.

Table 5. Partitioning total and explained variance in periphyton signatures by spatial scale (small (local, ≤~10 m), medium (10–500 m), and large (>500 m)) increments within the six study reaches of the main Ste. Marguerite River.

	Spatial scale		
	Small	Medium	Large
Total variance in increment	0.99	1.85	4.54
Variance explained by velocity and chlorophyll *a*	0.12	1.30	0.00
R^2	0.12	0.70	0.00

Note: At the local (small) scale, individual samples were standardized by subtracting site means. At the medium scale, site means were standardized by subtracting means of 500 m groupings. At the largest scale, 500 m group means were used.

SMR, mean signatures were the most enriched, and this signature gradient continues seaward into the brackish waters of the Ste. Marguerite Bay, where $\delta^{13}C$ signatures are −16‰ to −18‰, and out into the marine waters of the Saguenay Fjord, where $\delta^{13}C$ signatures are −12‰ to −15‰ (Morinville and Rasmussen 2006*b*).

There are also a number of other variables not measured in this study that might have affected the isotopic signatures observed and thereby contributed to residual variance in our models. Although shading by shoreline vegetation is minimal along the main river, the light regime may have affected the periphyton signatures and standing stock within the smaller tributaries. Two previous studies (MacLeod and Barton (1998) and Wienke and Fisher (1990)) had detected more enriched $\delta^{13}C$ signatures at well-illuminated sites. The effect of chlorophyll might also be underestimated if periphyton had been recently abraded by spates (Hill and Middleton 2006) or heavily grazed upon by benthos (Feminella and Hawkins 1995; Steinman 1996). We do not have the data to test it, yet it would be useful to examine possible effects of grazers on periphyton signatures. Differences in algal photosynthetic mechanisms can also affect primary producer $\delta^{13}C$ (Fogel et al. 1992). Fry and Wainright (1991) have shown that cell size influences marine phytoplankton $\delta^{13}C$. Species composition has also been suggested to increase epiphyte $\delta^{13}C$ variability (Osmond et al. 1981). Therefore variability in the composition of the benthic community might have contributed to residual variability in our models.

Explained variability: $\delta^{15}N$ versus $\delta^{13}C$

In most reaches, the variability of $\delta^{15}N$ was much less than that of $\delta^{13}C$, in many cases less than half. The variation of $\delta^{15}N$ was about the same as the residual variation of $\delta^{13}C$ after velocity and biomass were accounted for. This could suggest that it is the greater responsiveness of $\delta^{13}C$ fractionation to boundary-layer effects (caused by flow and the periphyton buildup) that makes it more spatially variable than $\delta^{15}N$. Although such velocity and biomass effects on $\delta^{15}N$ were not detected in this field study, we did detect a significant velocity effect on periphyton $\delta^{15}N$ in our laboratory experiment. Moreover, Finlay et al. (2002), in their field study on a California stream, also found a relationship between algal $\delta^{15}N$ and velocity very similar to that shown in our lab experiment. Failure of $\delta^{15}N$ to reflect the effects of velocity and biomass in the SMR could result from uniform boundary-layer depletion of N to the extent that no fractionation occurred, and therefore, variation in exchange flux made no difference. Thus spatial variabil-

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ity associated with velocity and biomass gradients at different scales that greatly enhance $\delta^{13}C$ variability would not be seen for $\delta^{15}N$. This idea receives some support from the fact that SMR has lower DIN concentrations (~4 μmol·L^{-1}) than those of the St. Lawrence River (DIN ~ 8 μmol·L^{-1} after Vis et al. 1998), which was the source of the water used in our laboratory velocity experiment (Trudeau and Rasmussen 2003). However, we were not able to test this hypothesis directly because we did not measure the $\delta^{15}N$ signature of the DIN in either the water column or the boundary layer.

An alternative explanation for our inability to explain $\delta^{15}N$ variation with the hypothesized predictors could be local variability in the N source signature from groundwater. Although highly heterogeneous N signatures in surface water and groundwater, of sufficient magnitude to override the velocity and biomass effects, have often been observed, they are usually anthropogenically induced and not seen in pristine areas such as the SMR (Heaton 1986; Cabana and Rasmussen 1996). This combined with the overall low within-reach variability of periphyton $\delta^{15}N$ makes this explanation doubtful.

Implications of spatial variation in periphyton signatures for food web studies

The pattern of spatial variation of periphyton stable isotope signatures may be passed on to consumers and potentially provide important information about either habitat factors (e.g., velocity or benthic productivity) or spatial information about consumer resource interactions. For example, Finlay et al. (2002) found different C and N signatures for invertebrates sampled in riffles than in pools, which allowed them to infer the feeding locations of both invertebrates and fish. Furthermore, enrichment of periphyton signatures in pools allowed separation of pool periphyton signatures from terrestrial detritus signatures. Morinville and Rasmussen (2003) were able to detect $\delta^{13}C$ signature differences between juvenile anadromous brook trout trapped during their outmigration and resident fish that remained behind. This signature difference indicated that the premigrant fish might be occupying faster water habitats than similar-aged residents, which was subsequently confirmed in a habitat study (Morinville and Rasmussen 2006*a*). In situations where the scale of movement of consumers is large (e.g., juvenile Atlantic salmon (*Salmo salar*)) and the consumers feed on invertebrates grown in pools, as well as in riffles, the consumer might be expected to average across and "smooth out" the variation spectrum of its resources. Although this type of smoothing tends to blur the signal of the environmental factors operating on the resources, it can potentially provide important information about the spatial scale of movement exhibited by the consumer, and we might expect to see variation further reduced at higher trophic levels if the scale of spatial averaging increases with trophic level. If in addition to riffle–pool differences there are large-scale patterns, such as the upstream–downstream gradient in periphyton $\delta^{13}C$ signatures seen in this study (see also Finlay 2001; Doucett et al. 1996), this could provide useful spatial information about higher trophic levels. For example, the signatures of outmigrating salmon smolts might link them to the river section in which they spent the most time. These are just a few ways in which spatial information contained in periphyton signatures could be useful in studies of higher-level consumers or in the analysis of the spatial scale of trophic interactions and of resource flows within ecosystems.

Sampling periphyton for stable isotopic signatures: how should the effort be allocated?

Our results indicate that roughly half of the within-reach variance in periphyton $\delta^{13}C$ is a result of the combination of water velocity and chlorophyll standing stock and their influence on fractionation via the boundary layer. In addition, this variability appears to be generated mostly via the meander-related pool–run–riffle sequences, which are manifested at different spatial scales in different stream systems. Superimposed on this is the even greater among-reach variance most likely attributable to source signature variations resulting from hydrological processes.

Thus, a few samples interspersed throughout the river system will provide a very poor estimate of the overall carbon signature of a river, a tributary, a reach, or even a small section of it. Even our study, which is based on a total of 264 $\delta^{13}C$ measurements of periphyton from eight reaches of the river system, cannot claim to have produced a very precise estimate of the average $\delta^{13}C$ signature for this river system. The estimate of within-reach variance for periphyton $\delta^{13}C$ obtained by pooling all data from this study was 2.67, and the corresponding pooled estimate for among-reach variance was 5.68, approximately twice the within-reach value. Our within-reach means are therefore quite precise; based on an average of 33 samples per reach, we obtain a pooled SE of only 0.3‰ (i.e., $(2.67 \cdot 33^{-1})^{0.5}$). On the other hand, because among-reach variance is much higher (5.68) and we sampled only eight reaches in the study, the pooled SE of our overall mean estimate of periphyton $\delta^{13}C$ for the whole river remains 0.89‰ (i.e.$[(5.68 \cdot 8^{-1}) + (2.67 \cdot 33^{-1})]^{0.5}$), assuming that within- and among-reach variances are independent and additive.

The among-reach variability was not a problem for our study, as the goal was to examine the relationships to our predictors, which were expressed mainly on a within-reach scale. However, if the goal had been to derive an average estimate of the periphyton $\delta^{13}C$ for the whole river system, broader sampling rather than intensive within-reach sampling would have been called for. Examples of situations in which larger scale averaging would be necessary are among-river comparative studies, either linking geochemical patterns to biota or perhaps attempting to link the signatures of anadromous fish otoliths to their river of origin. Any study of food web interactions will require a periphyton signature averaged over the appropriate scale of the consumer–resource interaction, which for some fish might be the local pool in which it spent most time. However, for some highly mobile fish, such as juvenile Atlantic salmon, the appropriate scale of signature required might extend well beyond a local reach perhaps to the whole-river scale.

To illustrate the precision gains from allocating sampling effort across many reaches, assume that our within- and among-reach estimates of variance are reasonable and that we had sampled 15 reaches of the river with only seven samples per reach. In this case, we would have obtained a standard error (SE) of estimate of 0.87‰ (i.e., $[(5.68 \cdot 15^{-1}) +$

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$(2.67 \cdot 7^{-1})]^{0.5}$), which is approximately the same as what we obtained from more than twice the number of samples (264 vs. 105). Because the among-reach variance is almost twice the within-reach value for the SMR, any optimum allocation of sampling effort would therefore require sampling about twice the number of reaches as the number of samples per reach. Within reaches, the maximum amount of variance seems to be generated at the 10–500 m scale with pool–run–riffle alternations, and thus, optimal sampling effort within reaches would ideally be oriented toward these features.

Although our models did not explain any of the periphyton $\delta^{15}N$ variance that we measured, our estimates of the average signatures for both the overall river and within reaches were much more precise for this isotope than for carbon, because the $\delta^{15}N$ variance was six times lower than $\delta^{13}C$ variance over the whole river system. Again, by pooling all data from the study, for $\delta^{15}N$ we obtain a within-reach variance estimate of 0.88, which is less than half of the corresponding value for $\delta^{13}C$, and an among-reach variance estimate of 0.47, more than an order of magnitude less than the corresponding estimate for $\delta^{13}C$. Thus, the SE of our reach means for $\delta^{15}N$ (based on an average of 29 samples per reach) was 0.16‰, and the overall SE of estimate was 0.3‰ $(= [(0.47 \cdot 7^{-1}) + (0.88 \cdot 29^{-1})]^{0.5})$, which are both much more precise than the corresponding $\delta^{13}C$ values. Because among-reach variance in this case is about half of the within-reach variance, optimal allocation of sampling effort would entail about twice as many samples taken within each reach as the number of reaches sampled. As no patterns were revealed regarding variability or correlations to other variables, samples could be taken essentially at random within reaches.

Acknowledgements

We thank Geneviève Morinville, David Browne, and other graduate students of the McGill University Biology Department for helpful suggestions on earlier versions of the manuscript. We also thank Laurence Piche for assistance in the field. The research was carried out at the CIRSA field station, and we are grateful for the opportunity to work there and for the support we received from the staff. We are especially grateful to James Luce and Michel Lapointe, Department of Geography, McGill University, for providing the GIS information for our study sites and for making a summary of their unpublished data on water chemistry and nutrients available to us. Financial support was provided by a Natural Sciences and Engineering Research Council of Canada (NSERC) Strategic Grant to JBR. We would also like to acknowledge the useful comments of two anonymous reviewers.

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Differential expression of corticosteroid receptor genes in rainbow trout (*Oncorhynchus mykiss*) immune system in response to acute stress

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Abstract: Expression of distinct corticosteroid receptor genes, glucocorticoid receptors 1 and 2 (GR-1 and GR-2, respectively) and mineralcorticoid receptor (MR), was quantified by real-time polymerase chain reaction (PCR) in peripheral blood leucocytes (PBL), spleen, and gill of rainbow trout (*Oncorhynchus mykiss*) after an acute netting stress. Plasma cortisol levels were significantly increased 2 h after stress and returned to prestress levels within 24 h. Consistent with changes in plasma cortisol, GR-2 mRNA levels in PBL increased significantly at 2 h after stress, returning to initial levels by 8 h. In contrast, GR-1 and MR levels in PBL decreased significantly at 24 h after stress, and these reduced levels were maintained for 7 days. Splenic mRNA levels of GR-1 and GR-2 also decreased at 8 h and 24 h after stress, returning to control levels by 7 days, whereas no significant change was observed in MR. In gill, there was no obvious change in corticosteroid receptor mRNA levels after stress, except for a transient decrease at 8 h in MR. These results suggest a variety of roles for the three corticosteroid receptors during immunosuppression in response to acute stress in trout.

Résumé : Nous avons mesuré l'expression de gènes distincts des récepteurs des corticostéroïdes, les récepteurs glucocorticoïdes 1 et 2 (GR-1 et GR-2 respectivement) et le récepteur minéralocorticoïde (MR), au moyen de la réaction en chaîne par polymérase (PCR) en temps réel dans les leucocytes sanguins périphériques (PBL), la rate et les branchies de truites arc-en-ciel (*Oncorhynchus mykiss*) après un stress aigu dû à la capture au filet. Les concentrations de cortisol plasmatique sont significativement plus élevées 2 h après le stress et elles retournent au niveau d'avant le stress en moins de 24 h. En accord avec les changements du cortisol plasmatique, les concentrations d'ARNm GR-2 dans les PBL augmentent significativement 2 h après le stress et sont retournées à leur niveau initial après 8 h. En revanche, les concentrations de GR-1 et de MR dans les PBL diminuent significativement 24 h après le stress; ces concentrations réduites se maintiennent pendant 7 jours. Les concentrations d'ARNm GR-1 et GR-2 dans la rate diminuent aussi 8 h et 24 h respectivement après le stress et retournent aux niveaux témoins en 7 jours; aucune changement significatif ne s'observe dans le cas de MR. Dans les branchies, il n'y a pas de changement observable des concentrations d'ARNm des récepteurs des corticostéroïdes après le stress, à l'exception d'une diminution passagère de MR à 8 h. Ces résultats laissent croire à l'existence d'une variété de rôles pour les trois récepteurs des corticostéroïdes durant l'immunosuppression en réaction à un stress aigu chez la truite.

[Traduit par la Rédaction]

Introduction

It is generally accepted that stress-induced secretion of cortisol leads to impairment of fish immune functions (Schreck 1996; Wendelaar Bonga 1997; Mommsen et al. 1999). Decreases in the number of antibody-producing leucocytes and in circulating levels of immunoglobulin M (IgM) were observed repeatedly in stressed or cortisol-administrated fish (Carlson et al. 1993; Hou et al. 1999; Saha et al. 2004). A rapid increase in plasma cortisol coincided with a decrease in the number of antibody-producing leucocytes in Chinook salmon (*Oncorhynchus tshawytscha*) that had been stressed by netting and confinement. These changes were associated with an increase in mortality caused by artificial infection with pathogen (Maule et al. 1986). According to Maule and Schreck (1990*a*), stress caused by netting or chronic confinement increased both the affinity and capacity of corticosteroid receptors, identified by specific binding with radiolabeled

Received 30 July 2006. Accepted 4 June 2007. Published on the NRC Research Press Web site at cjfas.nrc.ca on 3 October 2007. J19450

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 doi:10.1139/F07-110 © 2007 NRC Canada

cortisol, in leucocytes obtained from kidney and spleen of coho salmon (*Oncorhynchus kisutch*). These findings indicate that inhibitory effects of stress on the fish immune system are mediated through receptor-specific mechanisms for cortisol.

It is also noted that cortisol is not only involved in stress response but also possesses other physiological functions. Cortisol is the main corticosteroid in teleosts and is known to regulate both carbohydrate metabolism and hydromineral balance (Wendelaar Bonga 1997; Mommsen et al. 1999). Cortisol is also thought to be involved in sexual maturation and metamorphosis in fish, including the parr–smolt transformation in anadromous salmonids (Bentley 1998; Mommsen et al. 1999).

Two distinct cDNAs for glucocorticoid receptors (GR) have been cloned and characterized in rainbow trout (*Oncorhynchus mykiss*): GR-1 encoding a protein of 758 amino acids and GR-2 encoding 669 amino acids (Ducouret et al. 1995; Bury et al. 2003). Binding studies with recombinant GR-1 and GR-2 indicate that those two receptors have a similar affinity for glucocorticoids (Bury et al. 2003). However, induction of in vitro transcriptional activity of the GR-2 gene requires a much lower concentration of cortisol than needed by the GR-1 gene (Bury et al. 2003). In rainbow trout, another corticosteroid receptor cDNA has been isolated that has an apparent homology with the mineralcorticoid receptor (MR) sequences in tetrapods (Colombe et al. 2000). In trout, more potent transactivation of MR is shown with 11-deoxycorticosterone than with cortisol, using the mammalian cell line transfected with the vector inserted with trout MR gene (Sturm et al. 2005; Prunet et al. 2006). On the other hand, MR is the most sensitive receptor for cortisol to stimulate transcriptional activity than the other two GRs, using the mammalian cells with trout GR or MR genes (Bury et al. 2003; Prunet et al. 2006). In vivo transcriptional regulations of GR and MR have not been clarified yet during the physiological processes of stress response in fish.

In this study, we examined changes in mRNA levels for the corticosteroid receptor genes expressed in peripheral blood leucocytes (PBL), spleen, and gills of the rainbow trout to clarify the differential roles of corticosteroid receptors during immunosuppression after an acute stress. Changes in plasma levels of cortisol and IgM and numbers of IgM-positive PBL were also examined after exposure to stress to help shed light on the possible role of the receptors.

Materials and methods

Fish

Rainbow trout were reared at the National Research Institute of Fisheries Science at Nikko for successive generations in outdoor concrete ponds supplied with a continuous flow of spring water at 10 °C under natural photoperiod. They were transferred to indoor 50 L rectangular tanks containing 35–40 L of water, supplied with spring water at 1.5 L·min^{-1} at 10 °C. There were six fish in each tank weighing about 100 g each; thus density was 15–20 g body weight·L^{-1}. The fish were maintained under a lighting regime of 14 h light – 10 h dark for at least 3 weeks before use and were fed a commercially prepared dry diet (Oriental Yeast Co. Ltd., Mihama, Chiba) at 2% of body weight once per day. Feeding was stopped 24 h before the experiment.

Experiments and sampling

To assess effects of stress on immune function and corticosteroid receptor expression, fish were acutely stressed by holding them in a dip net out of water for 30 s, after which they were returned to their original tanks and sampled at 2, 8, and 24 h after stress. Control fish without stress were sampled at 0 h. Separately, fish stressed as described above and unstressed control fish were sampled at 1, 3, and 7 days. All fish in each tank were caught at the same time and anesthetized in tricaine methanesulfonate (MS-222, 0.15 g·L^{-1}; Sigma-Aldrich Corp., St. Louis, Missouri) buffered with $NaHCO_3$ (0.2 g·L^{-1}). Blood was collected from caudal vessels using a heparinized syringe in less than 5 min after the onset of anesthetization. Fish were then decapitated, and gills and spleen were sampled within 10 min.

PBLs were isolated as described previously (Yada et al. 2004). Briefly, blood was separated into two aliquots. One aliquot was centrifuged at 5000*g* for 5 min, and the plasma was stored at –80 °C until measurement of cortisol and IgM levels. The other aliquot was diluted 1:2 in minimum essential medium with Eagle salt (Sigma-Aldrich Corp.) containing 0.2% heparin sodium and buffered with 7.5% $NaHCO_3$ (pH 7.6). The mixture was placed on a 54% Percoll (Amersham Biosciences, Piscataway, N.J.) cushion and centrifuged at 400*g* for 25 min. The leucocyte band was harvested, washed with phosphate-buffered saline (pH 7.6), and centrifuged at 500*g* for 5 min. Contamination with erythrocytes was less than 1%. The isolated PBL fraction was separated into two aliquots, one of which was frozen in liquid nitrogen immediately and stored at –80 °C until use for extraction of mRNA. Spleen and gills were also frozen in liquid nitrogen and stored at –80 °C until mRNA extraction.

Flow cytometry

The other aliquot of PBL was used for flow cytometry with a murine monoclonal antibody to trout IgM, "1.14" (DuLuca et al. 1983). Leucocytes isolated as described above were stained with the 1.14 and a goat anti-mouse fluorescein isothyocyanate (FITC) labeled immunoglobulin G (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and then were then counted with a flow cytometer (EPICS PROFILE-II; Beckman Coulter, Inc., Fullerton, California). "9E1", a monoclonal antibody to channel catfish (*Ictalurus punctatus*) IgM, was used for negative control (Miller et al. 1998).

Plasma analyses

Plasma cortisol concentrations were determined by a radioimmunoassay as described by Redding et al. (1984). Plasma levels of IgM, which is the major type of Ig in teleost fish, were estimated by a specific enzyme-linked immunosorbent assay as described by Nagae et al. (1993). Optical density was measured with a microplate reader (SpectraMax 190; Nihon Molecular Devices, Tokyo, Japan).

RNA extraction and real-time polymerase chain reaction (PCR)

Total RNA was extracted from tissues and cells by the guanidium–isothiocyanate–phenol method (Chomczynski and Sacchi 1987) and treated with RNase-free DNase I (Takara Bio Inc., Shiga, Japan). After inactivation of DNase, reverse

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Table 1. Design of primers for cloning of standard cDNAs.

	Forward primer	Reverse primer
GR-1	5′-GACACCCCCAGATTTGTTAGATTAA-3′	5′-CTTTATCCATGGGTGAGAAGTTCTC-3′
GR-2	5′-TCAAACAGGAGAACACGTCTGC-3′	5′-TCCCTGCCAGGAGACACTTG-3′
MR	5′-TGTGCCGGAAGAAATGACTG-3′	5′-CCTCGTTGAAGACGAGGTCG-3′

transcription was carried out using SuperScript First-Strand Synthesis System (Invitrogen Corp., Carlsbad, California).

To determine absolute amounts of mRNA encoding the three receptors, partial cDNAs of rainbow trout GR-1, GR-2, and MR were cloned, sequenced, and used as standards. The GR-1, GR-2, and MR fragments were amplified from rainbow trout liver cDNA with AmpliTaq Gold DNA polymerase (PE Applied Biosystems, Foster City, California) and primers listed in Table 1. The amplification regime was 30 cycles consisting of 94 °C for 1 min, 56 °C for 30 s, and 72 °C for 1 min. The products were purified and subcloned into pT7 Blue (Novagen, Madison, Wisconsin). Sequences were certified using a 310 DNA sequencer (PE Applied Biosystems) as described by Ducouret et al. (1995), Colombe et al. (2000), and Bury et al. (2003). Cloned cDNA fragments were digested at the ends of inserts, separated by agarose gel electrophoresis, and purified using GENECLEAN (Qbiogene, Morgan Irvine, California).

Real-time PCR was performed with an ABI Prism 7900HT Sequence Detection System (PE Applied Biosystems). The PCR mixture (20 μL) contained 1× TaqMan Universal PCR Master Mix (PE Applied Biosystems), 300 nmol·L^{-1} each of forward and reverse primers, 100 nmol·L^{-1} of fluorogenic probe, and either standard ($6 \times 10^2 - 6 \times 10^7$ copies·reaction^{-1}) or product of reverse-transcribed RNA sample (0.04–40 ng·reaction^{-1}). After denaturation at 95 °C for 10 min, amplification was carried out by 50 cycles of 95 °C for 15 s and 60 °C for 1 min. The sequences of primers and probes in the assays are shown in Table 2. No amplification was observed with mismatched primer and probe sets. In each assay, standard cDNAs and assay samples were analyzed in triplicate. The intra- and inter-assay coefficients ($n = 4$) were 4.5% and 7.3% for GR-1, 3.2% and 10.6% for GR-2, and 1.1% and 7.0% for MR, respectively. Quantification of β-actin mRNA was carried out as described previously (Yada et al. 2005).

Statistical analyses

Significance of differences between two groups was analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test for parametric groups or Mann–Whitney *U* test for nonparametric groups. Calculations were performed using STATISTICA (Statsoft Inc., Tulsa, Oklahoma).

Results

Plasma cortisol levels were increased significantly at 2 h and 8 h after an acute stress, produced by netting and holding for 30 s, and then decreased to initial levels at 24 h (Fig. 1). On the other hand, plasma IgM levels and numbers of IgM-positive PBL decreased significantly 24 h after stress, although there was no significant change after 2 and 8 h.

Changes in mRNA levels are shown for GR-1, GR-2, and MR in PBL, spleen, and gill after stress (Fig. 2). In PBL, GR-2 mRNA levels increased significantly at 2 h after stress and returned to initial levels by 8 h. No further change was observed in GR-2 mRNA levels in PBL until after 24 h. In contrast, there were significant reductions in GR-1 mRNA levels at 24 h and in MR mRNA levels at both 8 and 24 h. In spleen, there were significant reductions in GR-1 mRNA at 8 h and in GR-2 at 24 h, whereas there was no significant change in MR. In gill, there was no obvious change in any type of receptor after stress, except for a transient but significant decrease in MR at 8 h. Initial levels of GR-1, GR-2, and MR mRNA expressed in PBL were significantly lower ($P < 0.01$) than those in spleen or gill. There was no significant difference in initial levels of GR-1 and MR mRNA between spleen and gill at 0 h, whereas GR-2 levels in gill were significantly higher ($P < 0.05$) than those in spleen.

The acute stress did not have any long-term effects, up to 7 days, on levels of plasma cortisol but did on the concentration of IgM and on the proportion of IgM-positive PBL (Fig. 3). Plasma IgM levels were significantly reduced at 1 and 3 days following stress. IgM-positive PBL reduced significantly 1 day after stress and then recovered to the control levels by 7 days.

GR-1 mRNA levels in PBL were significantly lower than the control at 1 and 7 days after acute stress (Fig. 4). Significant reductions in MR mRNA levels were also observed at 1, 3, and 7 days. In contrast, there was no significant change in levels of mRNA of GR-2. There was no significant difference in mRNA levels of GR or MR between the initial and control levels in PBL. In spleen, the decreased levels in GR-1 and GR-2 mRNAs were returned; there was no significant difference between stressed and control fish at 7 days. There was no significant effect of stress on MR gene expression in spleen. Although there was no significant difference between the initial and control levels in spleen 1 day after stress, there were significant tendencies to increase in GR-1, GR-2, and MR levels in the controls at 3 and 7 days when compared with those at 1 day ($P < 0.05$). In gill, there was no significant change in any of the receptors at 1, 3, or 7 days after acute stress.

Discussion

We found differential effects of acute netting stress on corticosteroid receptor genes in PBL, spleen, and gill of rainbow trout. GR-2 mRNA levels in PBL increased markedly at 2 h after stress and returned to initial levels by 8 h; no further change was observed until day 7. In contrast, gene expression of GR-1 and MR in PBL decreased significantly and levels were still low at 7 days after stress. The differential changes in corticosteroid receptor mRNA levels were also observed in spleen, an important lymphoid organ for antigen processing in teleosts, where GR-1 and GR-2 mRNA levels were decreased at 8 h or 24 h after stress and returned to control levels by day 7; there was no significant change in

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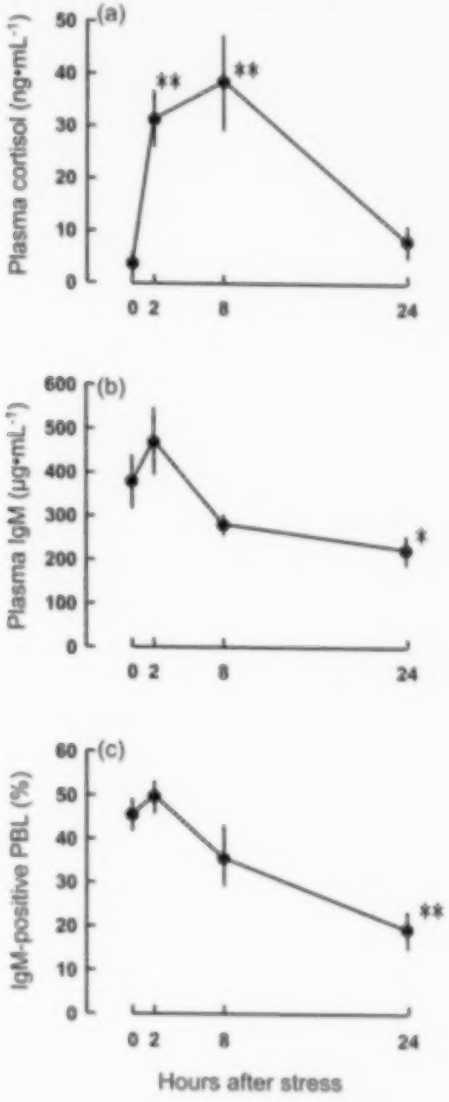
Table 2. Design of primers and fluorogenic probes for real-time PCR.

	Forward primer	Reverse primer	Probe
GR-1	5'-CGCAGCAGAACCAACAGTTG-3'	5'-ATGAGGGCGTCCAAGTACAGA-3'	5'-TGAGAACTTTGCTCTGTTGGAGGCGAGTAT-3'
GR-2	5'-TCCAGCAGCTATGCCAGTTCT-3'	5'-TTGCCCTGGGTTGTACATGA-3'	5'-AAGCTTGGTGGTGGCGCTG-3'
MR	5'-AGCTGATTGAGCCAGAGGTAGTG-3'	5'-CAGGTGGTCGGTGGTGTCA-3'	5'-TTGCGGGCTACGACAACACCC-3'

Fig. 1. Plasma (*a*) cortisol and (*b*) immunoglobulin M (IgM) levels and (*c*) number of IgM-positive peripheral blood leucocytes (PBL) at various times up to 24 h after netting and stress for 30 s. Data are expressed as means ± standard error of the mean ($n = 6$). Asterisks (*, **) denote significantly different from the initial level at $P < 0.05$ and 0.01, respectively.



MR. Interestingly, acute netting stress produced no obvious change in the three corticosteroid receptor genes expressed in gill other than the slight but significant decrease in MR levels at 8 h after stress. Differences in gene expression of corticosteroid receptors between PBL and spleen may relate to their differential roles in mediation of stress and the subsequent immunosuppression as indicated by decreases in plasma IgM levels and IgM-positive PBL.

A rapid increase in plasma cortisol levels observed at 2 and 8 h was followed by reduced levels of plasma IgM and IgM-positive PBL for 24 h. In rainbow trout, approximately

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Fig. 2. GR-1 (circles), GR-2 (triangles), and MR (squares) mRNA levels in (*a*) peripheral blood leucocytes (PBL), (*b*) spleen, and (*c*) gill at various times up to 24 h after netting and stress for 30 s. The mRNA levels for the two GR and MR were standardized by β-actin levels of each sample and expressed as mol·mol^{-1} β-actin. Data are expressed as means ± standard error of the mean (n = 6). Asterisks (*, **) denote significantly different from the initial level at $P < 0.05$ and 0.01, respectively.

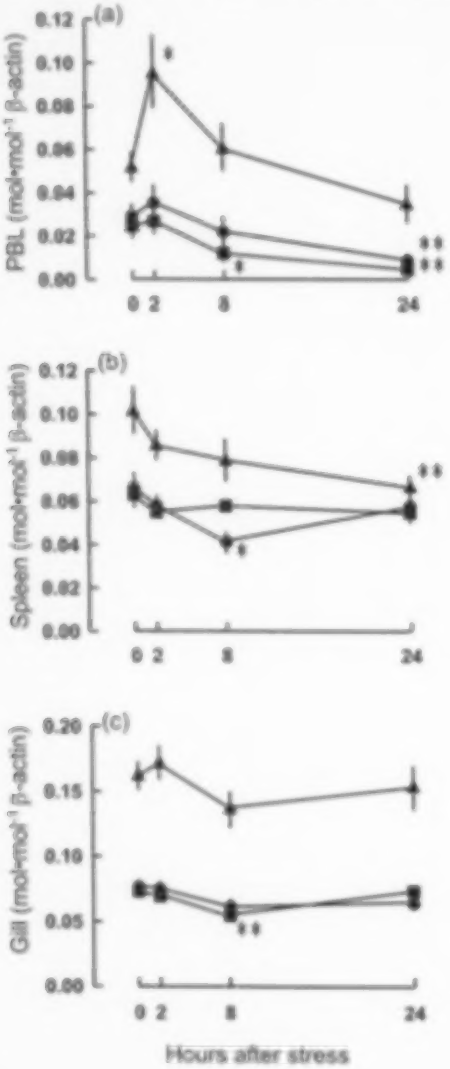
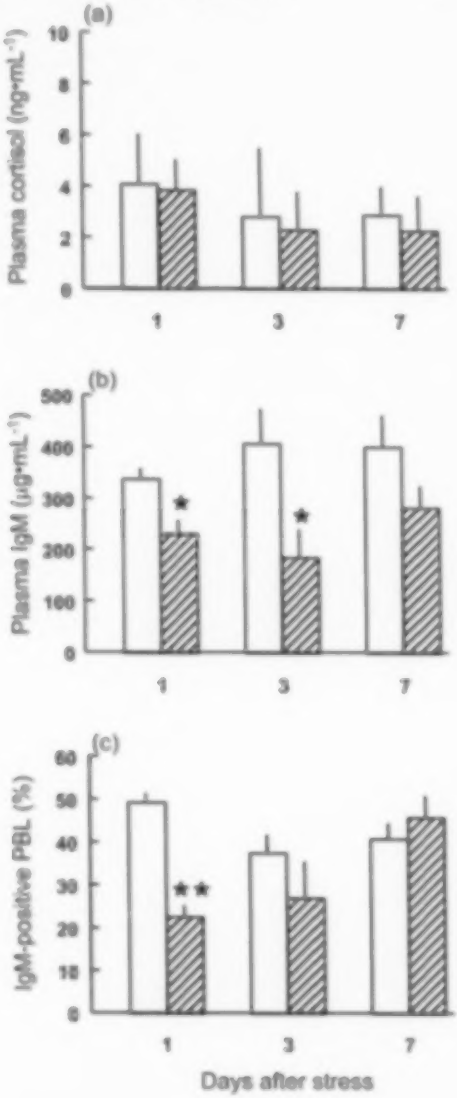


Fig. 3. Plasma (*a*) cortisol and (*b*) immunoglobulin M (IgM) levels and (*c*) number of IgM-positive peripheral blood leucocytes (PBL) at various times up to 7 days after netting and stress for 30 s (hatched column). Data are expressed as means ± standard error of the mean (n = 6). Asterisks (*, **) denote significantly different from the control level (open column) at corresponding time at $P < 0.05$ and 0.01, respectively.



90% of IgM-positive PBL are B-lymphocytes, which are the sites of production of circulating IgM (Manning and Nakanishi 1996). The decrease in IgM-positive leucocytes may reflect a decline in the number of PBL, as observed in stressed or cortisol-treated coho salmon (Maule and Schreck 1990*b*). The inhibition of antibody production after stress or cortisol administration has been observed in several fish species (Schreck 1996; Wendelaar Bonga 1997; Mommsen et al. 1999). Thus, the decrease in IgM-positive PBL in this study is likely caused by an increased secretion of cortisol following stress.

The rapid increase in GR-2 mRNA levels in PBL coincided with an elevation in plasma cortisol, suggesting an upregulation in the transcription of the GR-2 gene by cortisol. In leucocytes isolated from spleen and head kidney of coho salmon, Maule and Schreck (1991) revealed that both the number of binding sites and the dissociation constant for the binding of radiolabeled cortisol showed rapid increases within

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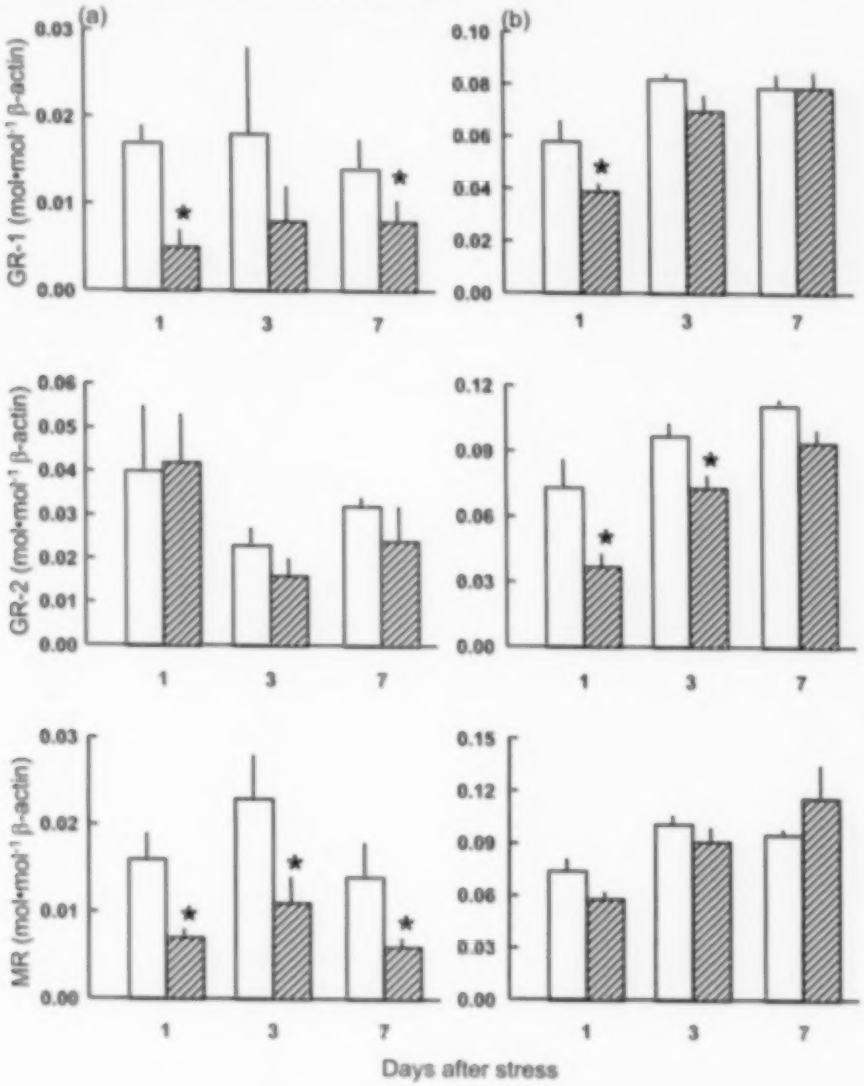
Fig. 4. GR-1, GR-2, and MR mRNA levels in (*a*) peripheral blood leucocytes (PBL) and (*b*) spleen at various times up to 7 days after netting and stress for 30 s (hatched column). The mRNA levels for the two GR and MR were standardized by β-actin levels of each sample and expressed as mol·mol^{-1} β-actin. Data are expressed as means ± standard error of the mean (n = 6). Asterisk (*) denotes significantly different from the control level (open column) at $P < 0.05$.



4 h after an acute netting stress, returning to control levels by 24 h. In carp (*Cyprinus carpio*), on the other hand, binding sites for corticosteroid in PBL decreased at 3 h after cortisol treatment (Weyts et al. 1998). In these kinetic studies, however, total binding of the corticosteroids to the three corticosteroid receptors was estimated. Inasmuch as GR-2 mRNA levels in PBL in the present study were two- to seven-fold higher than those for GR-1 and MR, it seems likely that there was a significant contribution of GR-2 for binding capacity to corticosteroid in PBL. We found that increases in plasma cortisol and in GR-2 expression were followed by long-term decreases in GR-1 and MR mRNA levels in PBL. Further studies are needed on the potential crosstalk among differential types of corticosteroid receptors in the process of immunosuppression.

Recently, Sturm et al. (2005) identified another MR in rainbow trout, which has 98.5% sequence homology with MR previously identified by Colombe et al. (2000). In vitro transactivation assays for the two MR using a mammalian cell line indicated that their transcription is similarly induced in the presence of cortisol (Sturm et al. 2005). We designed the probe and primers at the conserved region between the two MR mRNAs for the real-time PCR analysis. We also prepared a DNA fragment of the second MR, and the quantified results of this second fragment coincided well with those of the first one (data not shown). Thus, mRNA levels

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for MR in the present study represent the sum of expression of the two forms of MR. Sturm et al. (2005) examined the tissue distribution of MR mRNAs in rainbow trout using different primer sets from this study and reported that there was no significant difference in MR levels between spleen and gill, which is in agreement with our findings. The reduced expression of MR observed in PBL may relate to a downregulation of osmoregulatory receptor after stress with high plasma cortisol levels (Colombe et al. 2000; Sturm et al. 2005; Prunet et al. 2006).

In teleostean fishes, the gill plays a major role in ionic regulation and is a site of action for cortisol as a mineralcorticoid in fish (McCormick 1995; Mommsen et al. 1999). In addition to cortisol, 11-deoxycorticosterone and aldosterone are thought to act as mineralcorticoids, especially in rainbow trout (Sturm et al. 2005; Prunet et al. 2006). An upregulation of corticosteroid receptors in gill has been observed repeatedly in seawater-acclimated fish as either increases in mRNA levels or in binding sites tested by means of radiolabeled ligands (Uchida et al. 1998; Dean et al. 2003; Scott et al. 2004). On the other hand, the gill is also involved in the trapping and processing of antigens in the induction of immune responses in fish (Zapata et al. 1996). Stress-induced changes in the endocrine system, including cortisol secretion, result in the impairment of a variety of gill functions and modulate disease susceptibility (Schreck 1996; Balm 1997). In coho salmon, either repeated handling or chronic confinement reduces the number of binding sites for corticosteroids in gill, suggesting a downregulation of receptors (Maule and Schreck 1991; Shrimpton and Randall 1994). In the present study, however, the fact that there was limited influence of stress on mRNA levels of corticosteroid receptors in gill even after an elevation of plasma cortisol levels suggests differential regulations between binding capacity and gene expression of corticosteroid receptors.

Circulating levels of cortisol provide a useful indicator of stress in fish (Donaldson 1981; Barton and Iwama 1991). On the other hand, the response of cortisol to an acute stress is generally pulsatile and followed by a return to initial or control levels within several hours (Donaldson 1981; Barton and Iwama 1991; Sumpter 1997). It is also to be noted that factors other than stress, e.g., nutritional state, osmoregulatory requirements, and the process of parr–smolt transformation in anadromous salmonids, can produce considerable variation in circulating cortisol levels (Barton and Iwama 1991; Wendelaar Bonga 1997; Mommsen et al. 1999). The significant tendencies to an increase in control mRNA levels in spleen at 3 and 7 days could imply a possible influence of fasting on the gene expression of corticosteroid receptors even in lymphoid organs. Although these factors are not necessarily immunosuppressive (Yada and Nakanishi 2002), further studies are needed to clarify their interactions with corticosteroid receptor genes. The current study demonstrated that influences of an acute netting stress for 30 s are still detectable in mRNA levels of corticosteroid receptors in the immune system of rainbow trout for up to a week, a long time after circulating cortisol has returned to prestress levels. Quantification of the expression of corticosteroid receptor genes in the immune system may be useful as an indicator of stress and in assessing presence of slight or unnoticed stressors in fish under husbandry conditions.

Acknowledgments

We are grateful to Professor G. Flik, University of Nijmegen, Professor B.M.K. van Kemenade, Wageningen University, Dr. P. Prunet, Institut National de la Recherche Agronomique, and Professor Y. Takei, University of Tokyo, for their encouragement and invaluable suggestions during this study. We are indebted to Professor C. Bayne, Oregon State University, for critical reading of the manuscript. We are also indebted to Mr. Kohji Muto, National Research Institute of Fisheries Science at Nikko, for his help in maintaining the fish in good condition. This study was supported in part by a grant-in-aid from the Ministry of Agriculture, Forestry and Fisheries, Japan, and the Japan Society for Promotion of Science for International Cooperation to T.Y., by the National Science Foundation (IOB04-17250, IOB05-17769, OISE04-36349), US Department of Agriculture, and the Pauley Foundation Summer Programs 2004 and 2005 to T.H. and E.G.G., and also by the US Geological Survey, Oregon State University, and the Oregon Department of Fish and Wildlife to C.B.S.

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Can the precision of bottom trawl indices be increased by using simultaneously collected acoustic data? The Barents Sea experience

Vidar Hjellvik, Dag Tjøstheim, and Olav Rune Godø

Abstract: Acoustic data are recorded continuously during the winter survey for demersal fish in the Barents Sea. This paper presents a method for using the information from the acoustic recordings between trawl stations in an attempt to increase the precision of the trawl estimate. The method is related to the double-sampling regression estimation, in which information from a frequently sampled auxiliary variable (e.g., acoustics) that is correlated with the main variable (e.g., trawl) is used for the purpose of increasing the precision in the estimate of the population mean of the main variable. The version presented here allows for additional explanatory variables and for autocorrelation in the main and auxiliary variables. However, when applied to the Barents Sea data, only a minor variance reduction is obtained. The main reasons for this are a high autocorrelation in the acoustic data and a relatively low correlation between trawl and acoustics on trawl stations. Another unexpected result is that the acoustic density during trawling is significantly higher than between trawl stations.

Résumé : Durant l'échantillonnage d'hiver des poissons démersaux de la mer de Barents, il se fait un enregistrement continu des données acoustiques. Notre travail présente une méthode pour utiliser les enregistrements acoustiques entre les stations de chalutage pour augmenter la précision des estimations obtenues par chalutage. La méthode est reliée à l'estimation par régression à double échantillonnage, dans laquelle l'information obtenue sur une variable auxiliaire fréquemment échantillonnée (par ex., les données acoustiques) qui est en corrélation avec la variable principale (par ex. les données de chalutage) sert à améliorer la précision de l'estimation de la moyenne de la population de la variable principale. La version que nous présentons permet l'insertion de variables explicatives additionnelles et l'existence d'autocorrélation dans les variables principale et auxiliaires. Cependant, son utilisation avec les données de la mer de Barents ne produit qu'une faible réduction de la variance. Les raisons principales en sont une forte autocorrélation dans les données acoustiques et une corrélation relativement faible entre les données acoustiques et celles de chalutage dans les stations de chalutage. Un autre résultat inattendu est que la densité acoustique durant le chalutage est significativement plus importante qu'entre les stations de chalutage.

[Traduit par la Rédaction]

Introduction

The standard bottom trawl surveys comprise the principal fisheries independent data source for estimating abundance of commercial demersal fish in European waters. These surveys use samples taken from very small areas (tow tracks) as *representative of much larger inhomogeneous areas (strata)*, which can lead to a high variance in the resultant index. It is now possible, and in many cases routine, to collect acoustic data simultaneously during the surveys. Combining trawl and acoustic data would be one of the most cost-effective ways of improving these abundance estimates. As an illustration, in the Norwegian winter survey in the Barents Sea, trawl hauls with a duration of 1.5 nautical miles (n.mi) are typically taken every 20 n.mi, whereas acoustic sampling is done continuously along the survey track.

It is an obvious idea (see, e.g., Ona et al. 1991; Cachera et al. 1999) to try to use the additional information in the acoustic data to increase the precision of the trawl estimate. This was the objective of the European Union (EU) funded project CATEFA (Combining Acoustic and Trawl surveys to Estimate Fish Abundance), in which several approaches were tried: GAMs (generalized additive models) (Beare et al. 2004), artificial neural networks (Neville et al. 2004), geostatistics (Bouleau et al. 2004), and fuzzy logic (Mackinson et al. 2005). However, little was achieved in terms of increased precision of the trawl index. The purpose of the present paper is to seek to explain and understand these results. This is done by using statistical techniques for combined sampling to demonstrate that the potential for an improvement in precision is, in fact, small. Our data are restricted to the Barents Sea winter survey, but we believe that the conclusions of the analysis have a more general validity because the results follow from features of the data that seem to be present in many combined acoustic and trawl surveys, i.e., variable and

Received 10 August 2006. Accepted 24 May 2007. Published on the NRC Research Press Web site at cjfas.nrc.ca on 9 October 2007.
J19479

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 doi:10.1139/F07-101 © 2007 NRC Canada

often weak correlation between trawl and acoustics in addition to high autocorrelation of acoustic samples. As a consequence of the analysis, it will also emerge how strong the dependence should be between these two variables to obtain substantial improvement.

Our approach is based on standard statistical methods for combining information from two sets of data. Such methods are essential for the technique of double sampling (Cochran 1977, chapter 12). We will utilize this technique but do not impose the somewhat restrictive finite population sampling designs assumed in that book. Instead, we use a model that is more conventional in one respect (based on an infinite population) but more general in another. The increased generality is obtained by allowing for data coming from stationary time series or transect series and by permitting additional explanatory variables. We will continue to use the appellation "double sampling", but now this will just refer to a situation in which an auxiliary variable (acoustic density) is measured together with a main variable (trawl catch) at all locations where the main variable is observed and, in addition, at many other locations. If the main and the auxiliary variables are correlated, the idea is that information from the additional samples of the auxiliary variable should increase the precision of the estimate of the main variable.

Material and methods

The Barents Sea winter survey

This is a combined bottom trawl and acoustic survey for cod and haddock that has been undertaken annually since 1981 (Jakobsen et al. 1997). As the data before 1997 have a lower resolution, only data from 1997–2002 have been used. The horizontal resolution of these data is 1 n.mi, and the vertical resolution is 10 m (surface-related layers). In addition, the acoustic density near the bottom is available as bottom-related layers with 1 m vertical resolution. Because the horizontal resolution of the acoustic data is 1 n.mi and a trawl station typically covers 1.5 n.mi, there is not an exact match between the area covered by the two sample types. A rough estimate of the acoustic density corresponding to each trawl station has been calculated using a weighted average of the acoustic samples that overlap with the trawl station. This is described in detail in Hjellvik et al. (2003) together with a method for converting the surface-related layers to bottom-related layers. In Hjellvik et al. (2003), the correlation between trawl catches and acoustic densities was found to be highest when the acoustic density was accumulated from the bottom up to about 50 m above the bottom, and this part of the acoustic density has been used here.

The trawl catches have been transformed into ENASC (equivalent NASC (nautical area scattering coefficient, $m^2 \cdot (n.mi)^{-2}$)) values as

$$\text{ENASC} = \sum_L [(C_L \sigma_L)/(d w_L)]$$

where C_L, σ_L, and w_L denote catch in number, scattering cross section, and effective fishing width, respectively, for length group L, and d is the towed distance (for details, see Hjellvik et al. 2003). An overview of the survey methodology is given in Jakobsen et al. (1997).

The correlation between trawl and acoustics is essential in combining them. As the data are very skewed (approximately lognormally distributed), outliers can heavily influence the correlation estimates, and all of the analyses are therefore carried out on log-transformed data.

Typically, trawl stations are taken every 20th n.mi. However, the representativeness of a catch can be destroyed by clay in the codend, torn net, or other uncontrolled factors. Such catches are not used in the analysis. Moreover, for logistic reasons, the number of acoustic samples between trawl stations may be different from 20. The survey transect and the distribution of the number of between-station samples for the 2002 survey is shown (Fig. 1).

Double sampling with independent data

Let T_i and A_i denote the trawl catch (ENASC) and acoustic density (NASC), respectively, at trawl station i (notation is given in List of symbols). We want to estimate expected trawl catch $\mu(T)$ per square nautical mile in a certain region of size Ω, say. The average trawl catch $\bar{T}$ is an obvious estimate of $\mu(T)$.

The idea of double sampling is that the more frequent between-station observations A_j can be used to increase the precision of $\bar{T}$ as an estimate of $\mu(T)$ supposing that A_i and T_i are correlated. First, we explain the effect of double sampling in the simple situation in which there is a standard linear regression relationship

$$(1) \qquad T_i = \alpha + \beta A_i + u_i, \quad i = 1, \ldots, n_{ON}$$

between A_i and T_i. Here $\{u_i\}$ are independent identically distributed error variables with expected value $E(u_i) = 0$ and n_{ON} is the number of trawl stations. We assume A_j, $j = 1, \ldots, n_{ON}$, to be independent and independent of $\{u_i\}$. Observations for T_i are available for $i = 1, \ldots, n_{ON}$, whereas for A_j we have observations $j = 1, \ldots, n_{ALL}$, where n_{ALL} is the number of acoustic samples and is considerably larger than n_{ON}.

To explain the essentials of the method in a simple setting, first, we assume that A_j, $j = 1, \ldots, n_{ALL}$ are independent. This assumption is clearly not realistic as the acoustic samples are close in time and in space. In addition, both A_j and T_i will be influenced by spatial trends. More realistic assumptions will be introduced in the next section. We are interested in the expected value $\mu(T)$ of T using a combined index estimate $\hat{\mu}(T) = I_c$ composed of both the n_{ON} joint observations of (T_i, A_i) at trawl stations and the n_{ALL} minus n_{ON} acoustic observations taken between stations. From eq. 1, we have $\mu(T) = \alpha + \beta\mu(A)$ and $\bar{T}_{ON} = \alpha + \beta\bar{A}_{ON} + \bar{u}_{ON}$, where $\bar{T}_{ON}$, $\bar{A}_{ON}$, and $\bar{u}_{ON}$ are averages of on-station observations. Subtracting we have

$$\mu(T) - \bar{T}_{ON} = \beta[\mu(A) - \bar{A}_{ON}] - \bar{u}_{ON}$$

Because $E(u_i) = 0$, the best estimate of the mean $\bar{u}_{ON}$ of the regression residuals is zero, and it follows that $\mu(T)$ can be estimated by

$$\hat{\mu}(T) = I_c = \bar{T}_{ON} + \hat{\beta}[\hat{\mu}(A) - \bar{A}_{ON}]$$

The expected value $\mu(A)$ is estimated by using all of the acoustic observations, and hence

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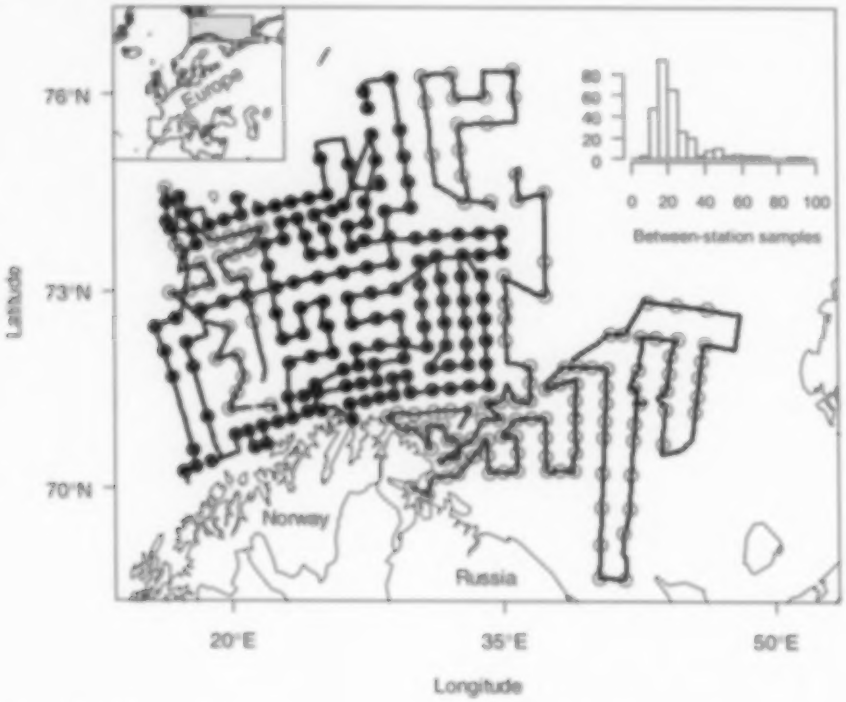
Fig. 1. Acoustic samples (small points, hardly distinguishable as they are so dense) and trawl stations taken by *Johan Hjort* (open circles) and *G.O. Sars* (solid circles) for the 2002 survey. Upper right corner: histogram of the number of acoustic samples between trawl stations for both vessels.



$$(2) \qquad I_c = \hat{\mu}(T) = \bar{T}_{\mathrm{ON}} + b_{\mathrm{ON}}(\bar{A}_{\mathrm{ALL}} - \bar{A}_{\mathrm{ON}})$$

where b_{ON} is the standard estimator of the regression coefficient β in eq. 1 based on the on-station joint observations.

Using standard properties of the regression model of eq. 1, the variance of I_C (see Appendix A for details) is

$$(3) \qquad \mathrm{var}(I_c) \approx (1-\rho_{A,T}^2)\frac{\mathrm{var}(T_i)}{n_{\mathrm{ON}}} + \rho_{A,T}^2\frac{\mathrm{var}(T_i)}{n_{\mathrm{ALL}}} \leq \frac{\mathrm{var}(T_i)}{n_{\mathrm{ON}}} = \mathrm{var}(\bar{T})$$

where $\rho_{A,T}$ is the correlation between trawl and acoustics. Note that eq. 3 is really an approximation (see Appendix A) that is only valid if n_{ON} is large (fulfilled in our case) and if $\{T_i\}$ and $\{A_j\}$ are series of "independent" samples from the survey area Ω. It will be generalized in the next subsection to take autocorrelation and possible explanatory variables into account. If T_i is measured in, e.g., kilograms per square nautical mile, ΩI_C is now an estimate of the total biomass in the survey area, and with the assumptions used in this subsection, it follows from eq. 3 that it will have lower variance than the trawl-only estimate if $\rho_{A,T} \neq 0$.

The correlation between trawl and acoustics is usually positive ($\beta > 0$), and hence usually $b_{\mathrm{ON}} > 0$, and the combined index I_C is adjusted upwards or downwards compared with the trawl-only index $\bar{T}$ according to whether or not $\bar{A}_{\mathrm{ALL}} > \bar{A}_{\mathrm{ON}}$.

Generalized double sampling

In a generalized version, trawl catches and acoustics are first modelled as regression functions of explanatory variables using the GAM (generalized additive model) approach, and the variance of the combined index (corresponding to eq. 3) is then calculated from the residuals of the regressions. This is done to take into account the explanatory power of covariables and to remove trends due to spatial variation, for instance. The residuals obtained from the chronologically ordered observations along the transect will be treated as time series. They are approximately stationary along the transect as opposed to the trawl and acoustic series themselves, which will contain trends. As long as the parallel sections of the transect are sufficiently far apart in time or in space, this will be true irrespective of the particular spatial sampling design chosen for the survey. The analogue for a trawl-only index is to fit a regression model and calculate the error of the predicted mean trawl catch from the regression error variance. The models fitted to the trawl and acoustic observations are

$$(4) \qquad T_i = f_T(x_{i1}, x_{i2}, \ldots, x_{ik}) + z_i, \quad i = 1, \ldots, n_{\mathrm{ON}}$$

$$(5) \qquad A_j = f_A(y_{j1}, y_{j2}, \ldots, y_{jl}) + \varepsilon_j, \quad j = 1, \ldots, n_{\mathrm{ALL}}$$

where f_T and f_A are functions of the explanatory variables $x_1, \ldots, x_k$ and $y_1, \ldots, y_l$ and where the residuals z_i, $i = 1, \ldots, n_{\mathrm{ON}}$, and ε_j, $j = 1, \ldots, n_{\mathrm{ALL}}$, are assumed to be stationary and autocorrelated. This autocorrelation signifies the continued presence of spatial dependence after trend removal along the transect. It is weak (from 0 to 0.4) for the trawl

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data, because the trawl stations are far apart and strong (0.8–0.95) for the acoustic data. The explanatory variables do not have to be the same for trawl and acoustics, although it seems reasonable that they are. Note that in fact we have two acoustic data sets, the entire set $\{A_j, j = 1,\ldots,n_{ALL}\}$ covering both trawl stations and the tracks between them and the on-station set $\{A_i, i = 1,\ldots,n_{ON}\}$. In the entire set, each observation is the NASC averaged over 1 n.mi, whereas in the on-station set, each observation is a weighted average of the two or three acoustic samples overlapping with the corresponding trawl station. For example, if trawl station i has a length of 1.5 n.mi and is covered by A_j (20%), A_{j+1} (100%), and A_{j+2} (30%), then $A_i = (0.2A_j + A_{j+1} + 0.3A_{j+2})/1.5$ (cf. Hjellvik et al. 2003). Acoustic residuals for the trawl stations are defined as

$$(6) \qquad e_i = A_i - f_A(y_{i1}, y_{i2}, \ldots, y_{ip}), \quad i = 1,\ldots,n_{ON}$$

where f_A is the function in eq. 5. In practice we use estimated residuals z_i, $\hat{e}_i$, and $\hat{\varepsilon}_j$ corresponding to estimates of f_T and f_A. Finally, as for eq. 2, the combined index is calculated as

$$(7) \qquad I_c = \bar{T}_{ON} + b_{ON}(\bar{\varepsilon}_{ALL} - \bar{e}_{ON})$$

where $b_{ON} = \hat{\beta}$ is the estimated regression coefficient in the regression $z_i = \alpha + \beta e_i + u_i$, $i = 1,\ldots,n_{ON}$, i.e., (z_i, e_i) are playing the role of (T_i, A_i) in eq. 1. Here, because these are on-station quantities, z_i, $i = 1,\ldots,n_{ON}$, e_i, $i = 1,\ldots,n_{ON}$, and u_i, $i = 1,\ldots,n_{ON}$, are weakly autocorrelated variables, and $\{e_i\}$ is assumed to be independent of $\{u_i\}$.

The index I_C of eq. 7 would in general be different from, but analogous to, the index I_C of eq. 2. If $\bar{\varepsilon}_{ALL} > \bar{e}_{ON}$, the acoustic residuals between stations are on average larger than those on station, and if $b_{ON} > 0$, as is usually the case, the combined index I_C is adjusted upwards as compared with the trawl-only index $\bar{T}$. Ignoring the uncertainty in fitting eqs. 4 and 5, i.e., in estimating f_T and f_A, and considering the explanatory variables (e.g., longitude and latitude) as nonrandom, it follows that $\text{var}(T_i) = \text{var}(z_i)$ and the variance of the trawl-only index is $\text{var}(\bar{z})$ (the variance of the mean error of eq. 4), whereas the variance of the combined index is given by $\text{var}(I_C) = \text{var}[\bar{z}_{ON} + b_{ON}(\bar{\varepsilon}_{ALL} - \bar{e}_{ON})]$. Using the derivation in Appendix A, we have

$$(8) \qquad \text{var}(I_c) = (1 - \rho^2_{e,z})\,\text{var}(\bar{z}_{ON}) + \beta^2\,\text{var}(\bar{\varepsilon}_{ALL})$$

where again this is an approximation based on the simplifying assumptions made above and in Appendix A. Note that in the case of independent z_is and ε_js, the index I_C of eq. 7 is completely analogous to I_C of eq. 2. Moreover, eqs. 8 and 3 are identical in this situation, which is easy to check using standard regression results. The change in variance obtained by the combined index eq. 7 compared with the trawl-only index $\bar{T} = \bar{T}_{ON}$ is

$$(9) \quad \Delta_V = 1 - \frac{\text{var}(I_c)}{\text{var}(\bar{T})} = 1 - \frac{(1 - \rho^2_{e,z})\,\text{var}(\bar{z}_{ON}) + \beta^2\,\text{var}(\bar{\varepsilon}_{ALL})}{\text{var}(\bar{z}_{ON})}$$

$$= \rho^2_{e,z} - \beta^2\frac{\text{var}(\bar{\varepsilon}_{ALL})}{\text{var}(\bar{z}_{ON})} = \rho^2_{e,z}\left(1 - \frac{\text{var}(\bar{\varepsilon}_{ALL})\,\text{var}(z_i)}{\text{var}(\bar{z}_{ON})\,\text{var}(e_i)}\right)$$

where we used $\beta^2 = \rho^2_{e,z}\,\text{var}(z_i)/\text{var}(e_i)$. Note that Δ_V will be small if $\rho^2_{e,z}$ is small.

The fact that ε_j, $j = 1,\ldots,n_{ALL}$, are autocorrelated must be taken into account when calculating $\text{var}(\bar{\varepsilon}_{ALL})$ in eq. 8. This can be done analytically or by autoregressive bootstrap if ε_j follows an autoregressive process, or by block bootstrap for more complicated ε_j processes like those of the survey data (see Appendix A for details). The calculation of $\text{var}(\bar{z}_{ON})$ can be done similarly, but here the first-order autoregressive analytic approximation is usually accurate because the autocorrelation generally is much weaker.

Checking the variance formula by simulation

The approach described in the preceding subsection consists of two stages: the fitting of a GAM regression to the data as in eqs. 4 and 5 and the application of the generalized double-sampling technique to the residuals from this fit, resulting in eqs. 7 and 8. As has been mentioned, there are some approximations involved in eq. 8. First, there are the approximations in the derivation in Appendix A. Second, the uncertainty has been ignored in the estimates $\hat{f}_A$ and $\hat{f}_T$ of f_A and f_T in eqs. 4 and 5 when computing $\text{var}(I_C)$ of eq. 8. We have therefore investigated the accuracy of the variance eqs. 8 and 9 by three sets of simulation experiments, two of them to some extent mimicking the 2002 survey. In all of the simulations, eq. A4 is used to calculate $\text{var}(\bar{z}_{ON})$, and the block bootstrap with block length 100 and 100 bootstrap replicates is used for $\text{var}(\bar{\varepsilon}_{ALL})$.

We simulate residual processes $\{z_j\}$ and $\{\varepsilon_j\}$ for trawl and acoustics, respectively, as first-order autoregressive processes (cf. Appendix A):

$$(10) \qquad \varepsilon_j = a\varepsilon_{j-1} + u_j + w_j,\ u_j \sim N(0, \sigma_u^2),\ w_j \sim N(0, \sigma_w^2), \qquad j = 1,\ldots,n_{ALL}$$

$$z_j = az_{j-1} + v_j + w_j,\ v_j \sim N(0, \sigma_v^2), \qquad j = 1,\ldots,n_{ALL}$$

where the sequences of independent normally distributed random variables $\{u_j\}$, $\{v_j\}$, and $\{w_j\}$ are independent. The correlation between acoustics and trawl is generated by the common variable $\{w_j\}$, where a higher value of σ_w^2 leads to higher correlation (cf. eq. A8 in Appendix A). For convenience, we first generate one trawl residual z_j for each acoustic residual ε_j, and then discard all of the z_js except the ones that correspond to on-station data. That is, we use the z_js with $j \in I_{ON}$, where I_{ON} denotes the set of samples that are on-station. Below we refer to this subset as $\{z_i\}$.

In the first experiment, we use $n_{ALL} = 5000$ and one trawl residual for each 20th acoustic residual, i.e., $I_{ON} = \{10, 30, \ldots, 4990\}$. This makes the simulated variance reductions directly comparable to theoretical variance reductions in eq. 9. Five values of a (0, 0.4, 0.6, 0.8, and 0.9) and four values of $\rho_{e,z}$ (0.3, 0.5, 0.7, 0.9) are used. Equations 7–9 are applied directly on the $\{z_i\}$ and $\{\varepsilon_j\}$ processes with no GAM involved, i.e., $\bar{T}_{ON}$ and $\bar{e}_{ON}$ in eq. 7 are replaced with $\bar{z}_{ON}$ and $\bar{\varepsilon}_{ON}$, respectively.

In the second experiment, the main change is that instead of using equally spaced trawl stations, we use the same spacing as in the 2002 survey. We use $n_{ALL} = 7383$ and $n_{ON} = 287$, as in the 2002 survey, and I_{ON} now denotes the set of samples for which A_i in the real data overlap with a trawl station (only one i for each station). The correlations and variances in the simulation are as for the demersal trawl and acoustic data in 2002, i.e., $a = \rho_\varepsilon(1) = 0.8673$, $\rho_{e,z} = 0.3841$,

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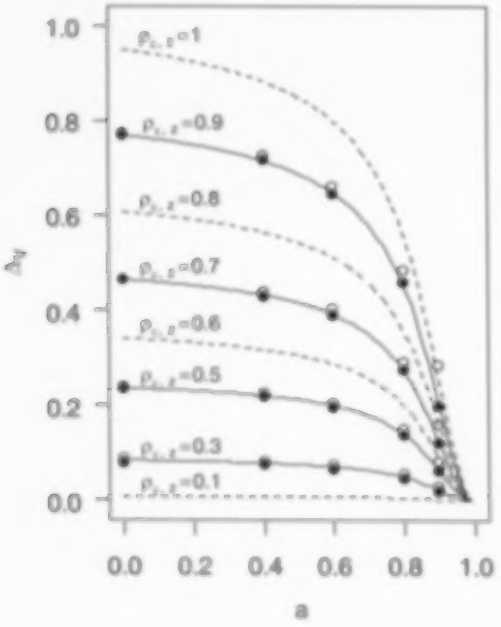
Fig. 2. Curves: theoretical variance reduction (Δ_V) calculated using eqs. 9 and A4, when the acoustic samples $\{\varepsilon_j, j = 1,\ldots,n_{ALL}\}$ and the trawl samples $\{z_i, i = 1,\ldots,n_{ON}\}$, $n_{ON} = n_{ALL}/20$, are first-order autoregressive processes as in eq. 10 with autocorrelations a and a^{20}, respectively, and the correlation $\rho_{\varepsilon,z}$ is as indicated in the figure. Solid and open circles are simulated variance reductions as described in the main text.



$\sigma_\varepsilon = 0.8116$, and $\sigma_z = 0.9664$, obtained by using $\sigma_u = 0.3917$, $\sigma_{u_\varepsilon} = 0.2517$, and $\sigma_{u_z} = 0.2992$ (cf. eq. A8 in Appendix A). This gives an autocorrelation of about 0.12 for on-station z_is.

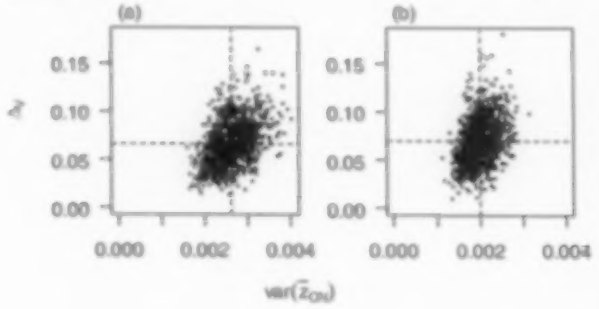
In the third experiment, in addition to the unequally spaced trawl stations, the GAM aspect is included, both in the data generation and in the index calculation. Acoustic samples A_j and trawl catches T_i are generated as

$$(11) \quad A_j = f_A(\text{lat}_j, \text{lon}_j) + \varepsilon_j, \quad j = 1,\ldots,n_{ALL}$$

$$T_i = f_T(\text{lat}_i, \text{lon}_i) + z_i, \quad i = 1,\ldots,n_{ON}$$

where the complete set $\{\varepsilon_j\}$ from eq. 10 is used to generate acoustic samples on and between stations, and $\{z_i\}$ (the on-station zs) from eq. 10 are used to generate trawl catches on stations. The functions f_A and f_T are taken to be the GAMs fitted to the 2002 data of demersal acoustics and catch, respectively, lat_i and lon_i are the latitudes and longitudes of the trawl stations that year, and lat_j and lon_j are the latitudes and longitudes of the acoustic samples. Next, a GAM was fitted to the simulated data, obtaining the estimates $\hat{f}_A$ and $\hat{f}_T$. The combined index and its variance were then calculated according to eqs. 7 and 8, replacing z_i by $\hat{z}_i = T_i - \hat{f}_T(\text{lat}_i, \text{lon}_i)$, $i = 1,\ldots,n_{ON}$, and ε_j by $\hat{\varepsilon}_j = A_j - \hat{f}_A(\text{lat}_j, \text{lon}_j)$, $j = 1,\ldots,n_{ALL}$, where again the latitudes and longitudes are taken from the 2002 survey.

Fig. 3. Variance reduction (Δ_V) as a function of $\text{var}(\bar{z}_{ON})$ for (*a*) simulation experiment 2 without any GAM involved and (*b*) experiment 3 with GAM involved. Broken vertical and horizontal lines show the average $\text{var}(\bar{z}_{ON})$ and variance reduction, respectively.



Results

Simulations

Because the interpretation of the results for the real data depends on the accuracy of eqs. 8 and 9, we present the results from the simulation experiments first (depicted in Figs. 2 and 3).

In the first experiment, the trawl samples $\{z_i, i = 1,\ldots,n_{ON}\}$ and acoustic samples $\{\varepsilon_j, j = 1,\ldots,n_{ALL}\}$ are described by the first-order autoregressive models of eq. 10, and the autocorrelations of $\{\varepsilon_j\}$ and $\{z_i\}$ are given by a and a^{20}, respectively. In this case, the theoretical variance reductions predicted by eqs. 8 and 9 are shown as curves (Fig. 2), with each curve having a fixed value for the correlation $\rho_{\varepsilon,z}$ between on-station trawl and acoustics. Equations A4 and A5 with the known values of a, $\sigma_\varepsilon^2 = \sigma_{u_\varepsilon}^2$, and $\sigma_z^2 = \sigma_{u_z}^2$ were used to calculate $\text{var}(\bar{\varepsilon}_{ALL})$ and $\text{var}(\bar{z}_{ON})$ in eq. 9. It is seen that the variance reduction decreases rapidly with increasing autocorrelation a for $a > 0.8$.

The results from the simulations in the first experiment are shown as points (Fig. 2). Each solid point shows the empirical variance reduction for 100 000 simulations for a given combination of autocorrelation a (0, 0.4, 0.6, 0.8, or 0.9) and trawl–acoustics correlation $\rho_{\varepsilon,z}$ (0.3, 0.5, 0.7, or 0.9). This is based on computing $\bar{z}_{ON}$ and $I_C = \bar{z}_{ON} + b_{ON}(\bar{\varepsilon}_{ALL} - \bar{z}_{ON})$ for each realization and then computing $\Delta_V = 1 - s_I^2/s_z^2$, where s_I^2 and s_z^2 are the empirical variances of the 100 000 I_Cs and $\bar{z}_{ON}$s, respectively. No approximation assumption or knowledge about the processes $\{\varepsilon_j\}$ and $\{z_i\}$ were used in the computations. The solid points are very close to the theoretical curves, demonstrating that the variance reduction formulas of eqs. 8 and 9 have high accuracy.

When working with real data, we have to estimate the quantities in eq. 9. Conventional estimates were used for $\rho_{\varepsilon,z}$ and β, and eq. A4 was used to estimate $\text{var}(\bar{z}_{ON})$ with $\text{var}(z_i)$ and a estimated from the data. The block bootstrap was employed to estimate $\text{var}(\bar{\varepsilon}_{ALL})$ in order to have a robust estimation procedure that is not dependent on a particular model chosen for $\{\varepsilon_j\}$. The estimation was carried out for each of 1000 simulated realizations (because $\text{var}(I_C)$ is much more time consuming to compute than I_C, Δ_V was only calculated for 1000 of the 100 000 realizations). For each realiza-

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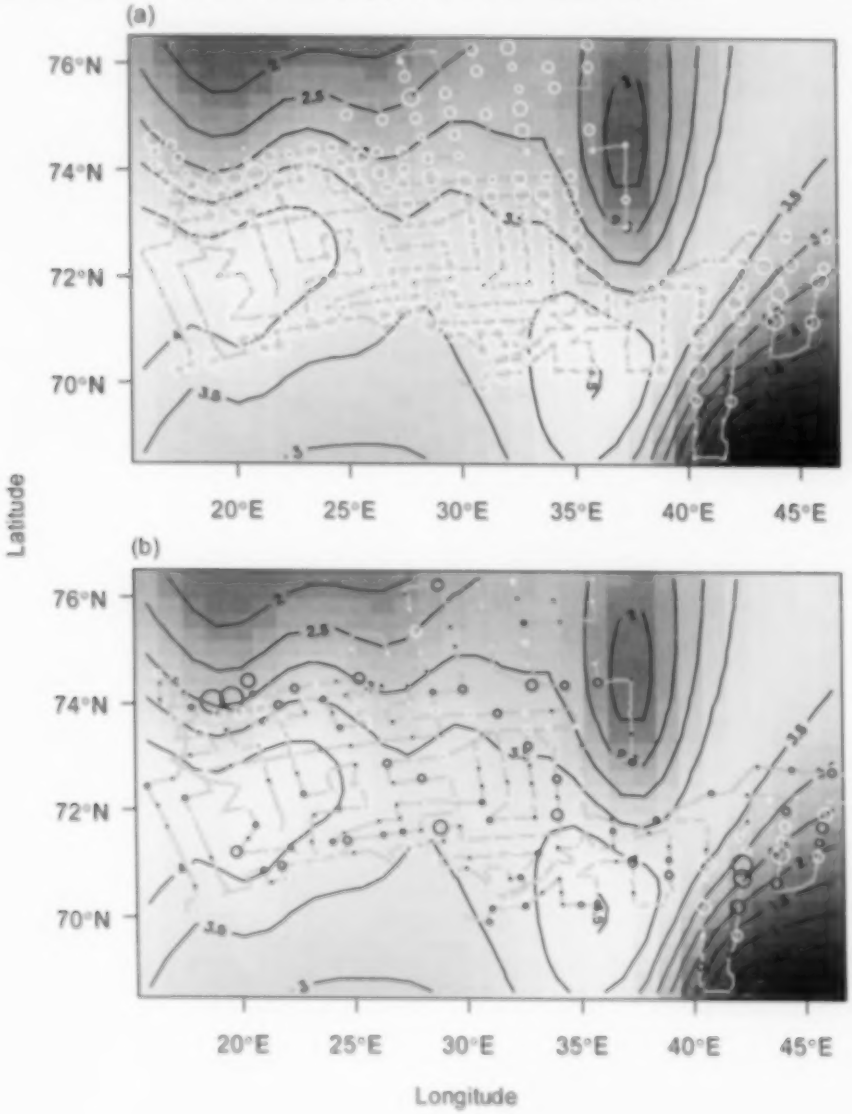
Fig. 4. The GAM fitted to demersal trawl catches in 2002. The background shading indicates the level of the fitted surface $f_I(\text{lon}_i, \text{lat}_i)$, with light shading corresponding to a high level. In (*a*), the log-transformed trawl catches are proportional to the diameter of the circles. In (*b*), the absolute value of the residuals from the GAM fit is proportional to the diameter of the circles. Black and white circles indicate negative and positive residuals, respectively, and the underlying grey lines indicate the survey track.



tion, the estimated variance reduction Δ_V was computed using estimated quantities in eq. 9 (the averages over the 1000 realizations are shown as open circles in Fig. 2). Again, we obtained a very good correspondence with the theoretical curves except for the combination of high as and high values of the correlation $\rho_{\varepsilon,2}$, where Δ_V was overestimated. If $\text{var}(\bar{\varepsilon}_{\text{ALL}})$ was calculated using the autoregressive bootstrap instead of the block bootstrap, there was no overestimation, but this is not a realistic approach for the real data. Increasing the block length to 500 and (or) the number of bootstrap replicas to 200 did not help. However, we do not think the overestimation represents much of a problem as we seldom observe pairs of values $(a, \rho_{\varepsilon,2})$ in the real data for which the overestimation in Fig. 2 is substantial.

The simulated variance reductions for the last two experiments mimicking the 2002 survey as described in the Material and methods section are shown (Fig. 3). On average, the variance reduction for the residual process with no GAM involved (Fig. 3*a*) is 0.067, whereas the theoretical variance reduction of Fig. 2 for the corresponding correlation and autocorrelation ($a = 0.876$ and $\rho_{\varepsilon,2} = 0.348$) is 0.041 when there is one trawl station for each 20th acoustic sample. However, with $n_{\text{ALL}} = 7383$ and $n_{\text{GIN}} = 287$, there is on average one trawl station for approximately each 26th acoustic

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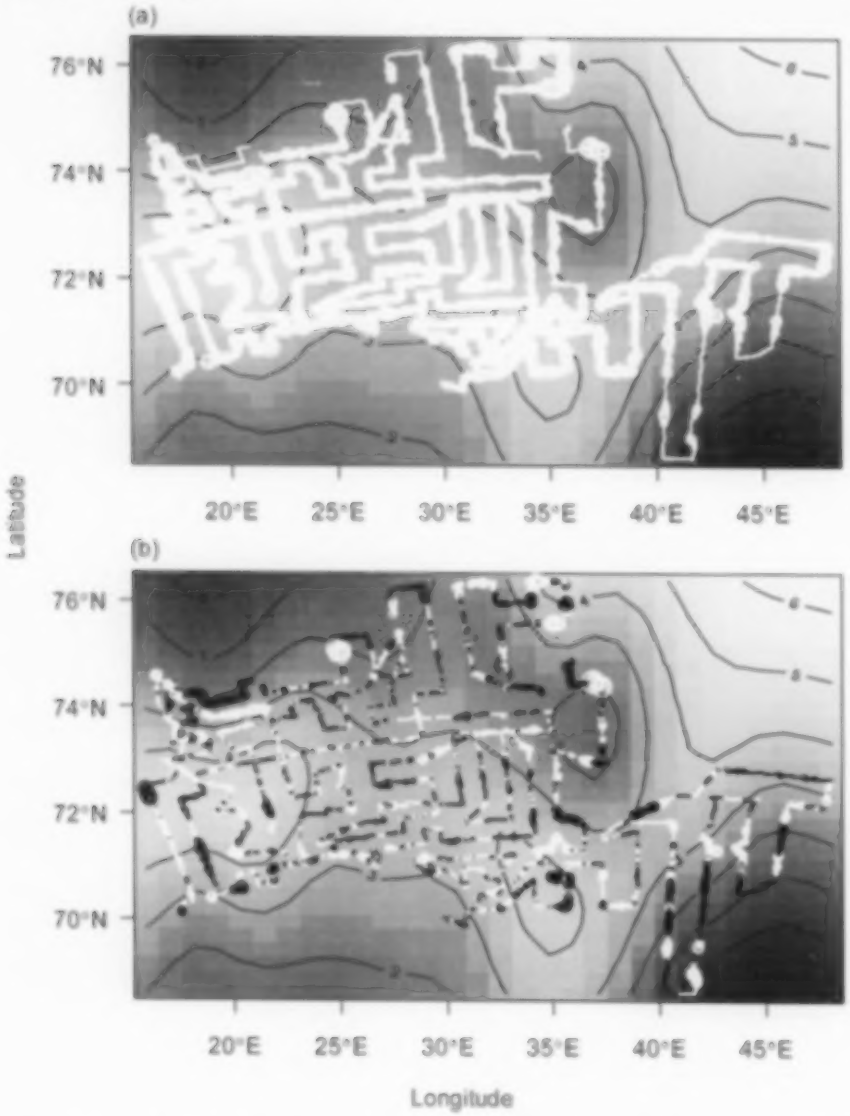
Fig 5. The GAM fitted to the demersal acoustics in 2002. The background shading indicates the level of the fitted surface $f_A(\text{lon}_j, \text{lat}_j)$, with light shading corresponding to a high level. In (*a*), the log-transformed echo abundance is proportional to the diameter of the circles. In (*b*), the absolute value of the residuals from the GAM fit is proportional to the diameter of the circles. Black and white circles indicate negative and positive residuals, respectively.



sample, which corresponds to a theoretical variance reduction of 0.055. The difference of 0.012 between this and the simulated variance reduction is as one would expect because of the tendency our method has to overestimate Δ_V for high autocorrelations (open circles in Fig. 2). Thus, the fact that the trawl stations are unequally spaced in experiment 2 (cf. the histogram in Fig. 1) does not seem to have much impact on the results. Including the GAM lead to a change in average Δ_V of only 5% to 0.070 (Fig. 3*b*).

We also did the simulations in the last two experiments with the first-order model of eq. 10 replaced by a 12th-order autoregressive model fitted to the 2002 demersal data. This resulted in a decrease of 0.01 in the average Δ_V both with and without the GAM included.

Real data

The data were log-transformed and modelled with latitude and longitude as explanatory variables, i.e.,

$$\log(T_i + 1) = f_T(\text{lat}_i, \text{lon}_i) + z_i$$

$$\log(A_j + 1) = f_A(\text{lat}_j, \text{lon}_j) + \varepsilon_j$$

where f_T and f_A are estimated nonparametrically by GAMs. A constant of 1 is added to the raw data in the log transfor-

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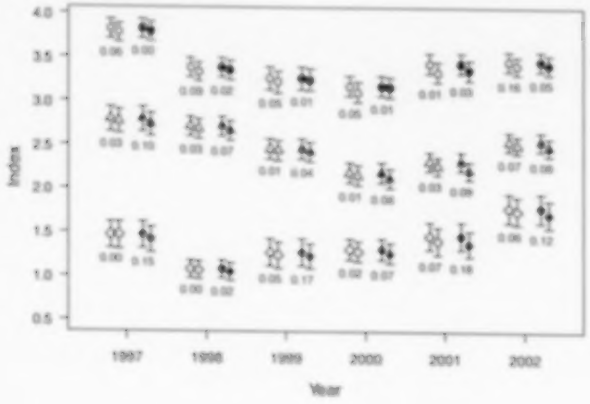
Fig. 6. Trawl-only indices (left in each pair) and combined indices (right in each pair) for demersal fish (circles), cod (triangles), and haddock (diamonds). Solid symbols indicate scrutinized acoustics (cod, in the case of demersal catch). The data are log-transformed. The error bars indicate ±2 standard deviations, calculated from eq. 9 for the combined index. The numbers indicate variance reduction.



mation because then zero observations remain zero observations after the transformation, and the residuals become approximately normally distributed. The GAM fit to the demersal trawl catches in 2002 is shown together with $\log(T_i + 1)$ and z_i (Fig. 4). The corresponding plots for the acoustics are also provided (Fig. 5). The contours of the surfaces are similar for trawl and acoustics, and for both variables, there is a tendency to have the largest residuals (in absolute value) close to the borders of the survey area. This is reasonable as the scarcity of data here makes the GAM fit less reliable. A careful study of Figs. 4 and 5 reveals that the GAM is quite effective in removing spatial trends, and actually, the GAM fit results in a variance reduction close to 50% for 2002 (cf. Fig. 8 in the Discussion). A strong autocorrelation along the transect remains for the acoustic residuals (Fig. 5).

In some of the acoustic series, there are periods with no fish and hence $\varepsilon_i = \varepsilon_{i-1}$. Because of this nonstationarity in the $\{\varepsilon_i\}$ series, block bootstrapping (see Appendix A) with 1000 replications and a relatively long block length of 100 was used to calculate $\mathrm{var}(\bar{\varepsilon}_{ALL})$, whereas eq. A4 was employed to calculate $\mathrm{var}(\bar{z}_{DN})$. The variance reduction Δ_V computed using eq. 9 was on average about 0.06, ranging from 0 to 0.18 (Fig. 6). For cod and haddock, in all years, Δ_V was largest when the scrutinized acoustics was used (0.10 on average), and for demersal fish, it was typically largest when the demersal acoustics was used. Note also that in almost all cases, the combined index has a lower value than the trawl-only index. The variance reduction clearly increases with the correlation between trawl and acoustics (Fig. 7*a*), but more unexpectedly, it also tends to increase with increasing autocorrelation (Fig. 7*b*). This seems to be because high correlation between trawl and acoustics tends to occur together with high autocorrelation in acoustics (Fig. 7*c*). For a fixed correlation, the variance reduction decreases with increasing autocorrelation.

Discussion

The variance reduction obtained for the Barents Sea bottom trawl survey estimate by using acoustics as an auxiliary variable in the combined sampling approach was relatively small (typically less than 10% on the log scale). This is due to the high autocorrelation in the acoustic residuals (which means that each acoustic sample does not carry much independent additional information) combined with a relatively low and variable correlation between trawl and acoustics, and the observed variance reductions are approximately as one would expect (Fig. 2). For a lag-one autocorrelation around 0.9, the variance reduction for first-order autoregressive processes is quite small for moderate correlations (Fig. 2). For higher-order autoregressive processes, the situation is even worse because the autocorrelation decreases less rapidly as the lag increases. For the Barents Sea data, the autocorrelation varies a lot between years and acoustic fish categories. The lag-20 autocorrelation varies from about 0.04 (demersal 1998) to about 0.41 (haddock 2002). The fact that low acoustic autocorrelation is generally associated with low correlation between trawl and acoustics and vice versa does not help. It may indicate that there is a latent variable or trend with which both the acoustics and trawl data are positively correlated.

Because the scrutinized acoustic density is more strongly correlated with the trawl catches than is the total acoustic density, one would expect the best results when using the former as an auxiliary variable. However, because the scrutinizing process is based to some extent on the species distribution in the trawl catches, there is, in principle, a possibility that the scrutinized acoustic density is biased (for example, if there is a high percentage of cod in the catch at a given trawl station, much of the acoustic echo abundance in the vicinity of that station may be allocated to cod). This bias could yield a too high trawl–acoustics correlation and hence a too high estimated variance reduction. Moreover, the scrutinized data have slightly higher autocorrelation. The use of total acoustic density would in this sense be safer.

The effect of simplifying assumptions

The time series approach is a simplification with regards to the Barents Sea data, because the spatial aspect is ignored. However, by considering the residuals after fitting a GAM, using latitude and longitude as explanatory variables, most of the large-scale spatial trends should be removed, and the autocorrelation along the survey transect is the dominating one. The reduction in total variation obtained by including latitude and longitude as explanatory variables is much larger than the additional reduction obtained by including acoustics (Fig. 8; see Mackinson et al. (2005) for corresponding results for North Sea surveys). Also, the combined index in eqs. 4–9 is computed for log-transformed data, yielding log indices, and it is not trivial to get a back-transformed combined index with an appropriate uncertainty measure. On the other hand, "real" trawl-only indices with uncertainty estimates can be calculated using the GAM approach, for example, in combination with bootstrapping.

Another simplification is the treatment of the acoustic residuals within one year as a contiguous time series, despite

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Fig. 7. Variance reduction (Δ_V) plotted against (*a*) correlation between trawl and acoustics and (*b*) autocorrelation in acoustic residuals and (*c*) autocorrelation plotted against trawl–acoustics correlation. Each point corresponds to one of the pairs in Fig. 6. Circles, triangles, and diamonds represent demersal fish, cod, and haddock, respectively. Open and solid symbols represent demersal and scrutinized acoustics (cod, in the case of demersal catch), respectively.

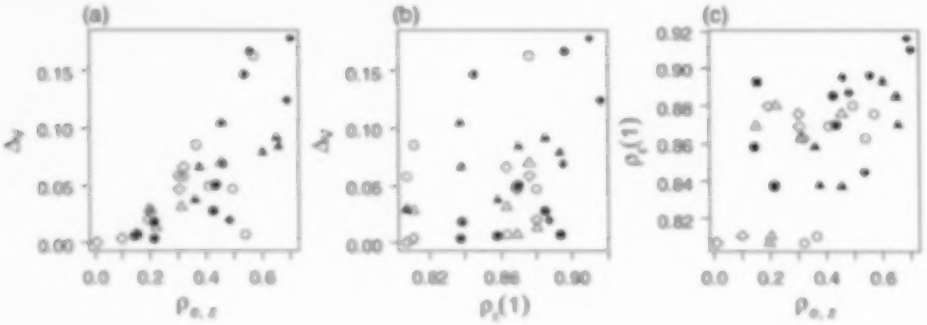
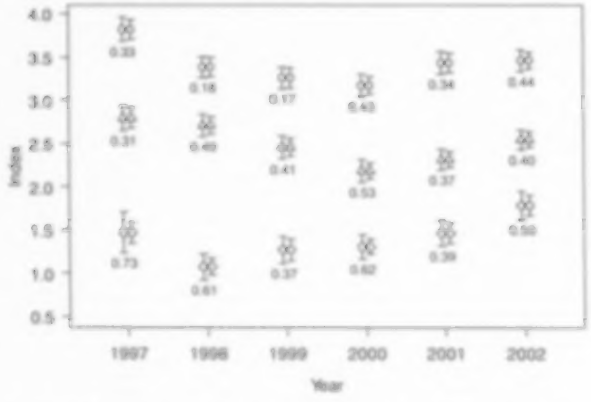


Fig. 8. Mean catch $\bar{T} \pm 2$ bootstrapped standard errors for demersal fish (circles), cod (triangles), and haddock (diamonds). The standard errors are calculated by bootstrapping the catches themselves (left in each pair) or residuals from a fitted GAM with latitude and longitude as explanatory variables (right in each pair). Numbers: variance reduction defined as $\Delta_V = 1 - \text{var}(\hat{\varepsilon})/\text{var}(\bar{T})$, where $\{\varepsilon_i\}$ are the residuals from the GAM fit.



the fact that two or three vessels are involved. There are typically some discontinuities in the series for each vessel as well. However, the number of discontinuities is small compared with the total number of samples, and taking these into account would probably not have improved the results.

Further, the on-station and between-station acoustic observations are not subsamples of the same process. The elementary sampling distance unit (EDSU) is 1 n.mi. and the between-station samples cover 1 EDSU each, whereas the on-station acoustic densities are weighted averages of two or three successive EDSUs overlapping the towed distance of 1.5 n.mi. However, setting the on-station acoustic density equal to the density of the first EDSU covered by the trawl station did not improve the variance of the combined index. Adjusting trawl catches and acoustic densities for diurnal variation did not lead to appreciable changes either. If the on-station acoustic abundance for each trawl station had been integrated exactly over the trawling distance, then the trawl–acoustics correlations would probably have been slightly higher, but it is dubious whether the variance reduction would increase much. By increasing the EDSU to, for example, 2 n.mi, one would obtain a lower autocorrelation in the acoustic data, but the number of acoustic samples would decrease correspondingly, so the variance reduction would not improve. The tendency of overestimation for high autocorrelations (cf. the simulation experiments) would, however, be smaller.

Our analysis is based on stationarity, and this assumption seems roughly to be fulfilled as regards cod and demersal fish, but in the haddock time series, there are periods with no fish ($A_j = 0$) and hence $\varepsilon_j = \varepsilon_{j-1}$. Removing these periods, however, did not influence the autocorrelation much.

On- and between-station differences

The properties of the acoustic residuals between stations are different from those on stations (Fig. 9). Overall, the variance is slightly higher between stations, whereas the means between stations are considerably lower. On average, the variance is about 7% higher between stations, and to check for the effect that this has on the estimated variance reduction, we did a simulation experiment in which the between-station εs in eq. 11 were multiplied by $\sqrt{1.07}$. For $\rho_{\varepsilon,\varepsilon} = 0.6$, this led to a reduction in Δ_V from 0.079 to 0.062.

In this study, we have seen that the combined index almost always yields a lower value than the trawl-only index. The reason for this is that the on-station means, particularly for *G.O. Sars*, are higher than the between-station means (Fig. 9; in the upper right plot, the between-station means are close to zero because $\hat{\varepsilon}_{\text{ALL}} = 0$ due to properties of the GAM, and $\hat{\varepsilon}_{\text{BETW}} = \hat{\varepsilon}_{\text{ALL}}$ because the on-station εs are few). Taken together with the lower variance on station, this indicates that during trawling the vessel (in particular *G.O. Sars*) may in some sense act as a fish-attracting device (cf. Røstad et al. 2006). The findings in Handegard and Tjøstheim (2005) support this hypothesis. An alternative explanation is that the behavior of fish alerted by, e.g., a trawling vessel will change towards a more aimed and coordinated swimming pattern resulting in a reduction in average tilt angle. The result will be an increased average target strength as is observed during trawling. It should be noted that this runs contrary to some earlier results (Ona and Godø 1990): based on 134 trawl stations from 1985 to 1986, taken by vessels other than *Johan Hjort* and *G.O. Sars*, the acoustic density

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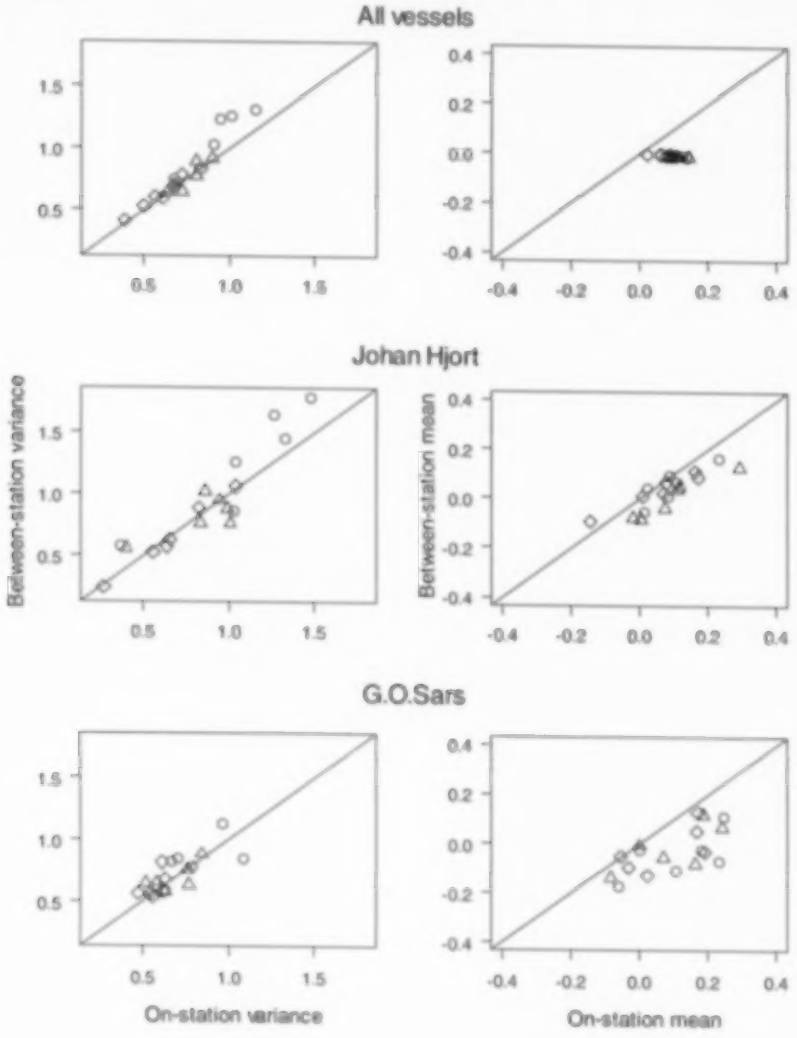
Fig. 9. On- and between-station variances (left plots) and means (right plots) of ξ_i. Each point corresponds to one of the acoustic categories and years in Fig. 6 (circles, triangles, and diamonds represent demersal fish, cod, and haddock, respectively). The solid lines indicate $y = x$ (equal variance and mean). In the upper plots, all samples from each survey are used; in the lower plots, only samples taken by *Johan Hjort* or *G.O. Sars* are used.



was lower during trawling than before or after. We consider the results illustrated in Fig. 9 to be important and requiring further investigation.

The higher on-station means support the findings of Bez et al. (2007). They demonstrated a generally high consistency between on-station and between-station acoustics for several surveys, including the Barents Sea winter survey, using tools like the variogram and a global index of collocation. However, the acoustic density in the bottom layers tended to be higher during trawling than just before or after trawling. In the current analysis, we have also used the acoustic density in the bottom layers (up to 50 m above the bottom), but for the whole water column the echo abundance for demersal fish is on average 12% lower before and after trawling than during trawling for *G.O. Sars* and 1% lower for *Johan Hjort* (N.O. Handegard and V. Hjellvik, unpublished data).

Alternative combination methods?

In conclusion, the precision of the Barents Sea bottom trawl survey index cannot be increased much by using the simultaneously collected acoustic data, at least not using standard statistical techniques as suggested in this paper. Moreover, from eqs. 9 and A4, such a result can always be expected in the case where one has high autocorrelation of the acoustics and quite low correlation between acoustics and trawl catches. It seems to be difficult to get rid of the autocorrelation for the acoustics. If there is an auto-

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correlation of 0.8, a correlation of about 0.9 is required between trawl and acoustics to obtain a 50% variance reduction (on the log scale if the data are log-transformed).

An alternative approach could be to employ the acoustic data to allocate trawl effort in an adaptive sampling design (Ona et al. 1991; Everson et al. 1996). Further, there is no doubt that the acoustic data do contain information not present in the trawl data. For example, the bottom trawl does not catch the fish distributed in the upper part of the water column, whereas they are detected by the acoustics. Thus, an intuitive way of combining trawl and acoustic data is to add the acoustic density above the effective fishing height of the trawl to the trawl catch. As is well known, this is not an easy task. The effective fishing height of the trawl is higher than the physical height of the trawl, because fish situated higher in the water column are to some extent disturbed and move downwards to the bottom where they are caught by the trawl. However, the details of this mechanism are not well understood (Handegard and Tjøstheim 2005; Hjellvik et al. 2003). Both survey methods are, for example, sensitive to diurnal variation and vertical distribution phenomena (Hjellvik et al. 2002, 2004), but such systematic influential factors may affect the relationship in density estimates in a manner that we are not yet able to take into account (Godø 1994). Also, the length and species composition of fish is typically not the same close to the bottom as higher in the water column. Using the composition in the trawl catch to allocate the acoustic backscatter to species and length groups will therefore bias the combined estimate. It thus appears that an efficient combination of trawl and acoustic estimates of density still needs improved quantitative understanding of the complex behavioural processes influencing trawl and acoustic efficiency.

Acknowledgements

We thank the European Union for financial support through the CATEFA (Combined Acoustic and Trawl data for Estimating fish Abundance) project (Q5RS-2001-02038). We are grateful for a number of very valuable suggestions and comments from the referees and an associate editor on an earlier version. In particular, the GAM fitting of all, not only the on-station part, of the acoustic data in eq. 5 is due to a suggestion from one of the referees.

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List of symbols

Symbol	Meaning
T	Trawl catch
A	Acoustic sample
n	Number of samples
i, ON	Subscripts used for on-station trawl and acoustics
j, ALL	Subscripts used for all acoustic samples
z	Residuals from regression model fitted to trawl catches (eq. 4)
ε	Residuals from regression model fitted to all acoustic samples (eq. 5)
e	On-station acoustic "residuals" (eq. 6)
β	True regression coefficient between trawl and acoustics
b_{ON}	Estimated regression coefficient between trawl and acoustics
$\rho_{X,Y}$	Correlation between X and Y
$\rho_X(k)$	Lag k autocorrelation of X
I_C	Combined index (eq. 7)
Δ_V	Variance reduction
L	Block length in block bootstrap
*	Superscript indicating bootstrap sample

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Appendix A

Derivation of eq. 3

Using eq. 1 twice and eq. 2, elementary algebra yields

$$\begin{aligned}(A1)\quad \hat{\mu}(T) - \mu(T) &= I_C - \mu(T)\\ &= \bar{T}_{ON} + b_{ON}(\bar{A}_{ALL} - \bar{A}_{ON}) - \alpha - \beta\mu(A)\\ &= \alpha + \beta\bar{A}_{ON} + \bar{u}_{ON} + b_{ON}(\bar{A}_{ALL} - \bar{A}_{ON}) - \alpha - \beta\mu(A)\\ &= \beta[\bar{A}_{ALL} - \mu(A)] + \bar{u}_{ON} + (b_{ON} - \beta)(\bar{A}_{ALL} - \bar{A}_{ON})\end{aligned}$$

When n_{ON} gets large, the last product term in the last line is clearly of smaller order and can be neglected as both $(b_{ON} - \beta)$ and $(\bar{A}_{ALL} - \bar{A}_{ON})$ go to zero approximately at the same rate as the first two terms. Because of the independence of A_j, $j = 1,\ldots,n_{ALL}$ and u_i, $i = 1,\ldots,n_{ON}$, with this approximation we can write

$$(A2)\quad \mathrm{var}(I_C) = \beta^2\frac{\mathrm{var}(A_j)}{n_{ALL}} + \frac{\mathrm{var}(u_i)}{n_{ON}}$$

(see, e.g., Hogg and Tanis 2006, p. 211). It is a well-known result in standard linear regression (see, e.g., Hogg and Tanis 2006, p. 211) that $\mathrm{var}(u_i) = (1 - \rho^2_{A,T})\mathrm{var}(T_i)$ and $\beta^2\mathrm{var}(A_i) = \rho^2_{A,T}\mathrm{var}(T_i)$, from which eq. 3 follows.

Derivation of eq. 8

For the derivation of eq. 8, we replace T by z, $\bar{A}_{ALL}$ by $\bar{\varepsilon}_{ALL}$, and $\bar{A}_{ON}$ by $\bar{e}_{ON}$ in the above derivation of eq. 3 and obtain from eq. A1 with $I'_C = \bar{z}_{ON} + b_{ON}(\bar{\varepsilon}_{ALL} - \bar{e}_{ON})$,

$$I'_C - \mu(I'_C) = \beta[\bar{\varepsilon}_{ALL} - \mu(\varepsilon)] + \bar{u}_{ON} + (b_{ON} - \beta)(\bar{\varepsilon}_{ALL} - \bar{e}_{ON})$$

Using exactly the same approximation as in the derivation of eq. A2 and using independence of $\{\varepsilon_i\}$ and $\{u_i\}$, we can write

$$\mathrm{var}(I_C) \approx \mathrm{var}(I'_C) = \beta^2\mathrm{var}(\bar{\varepsilon}_{ALL}) + \mathrm{var}(\bar{u}_{ON})$$

If the on-station observations are taken so far apart that they can be considered independent, the standard result (Hogg and Tanis 2006, p. 211) quoted in the derivation of eq. 3 holds, i.e.,

$$(A3)\quad \mathrm{var}(\bar{u}_{ON}) = \frac{\mathrm{var}(u_i)}{n_{ON}} = (1 - \rho^2_{e,z})\frac{\mathrm{var}(z_i)}{n_{ON}}$$

If $\{u_i\}$, $\{e_i\}$, and $\{z_i\}$ are allowed to be weakly autocorrelated, but with $\{u_i\}$ independent of $\{e_i\}$, eq. A3 can be replaced by

$$\mathrm{var}(\bar{u}_{ON}) \approx (1 - \rho^2_{e,z})\,\mathrm{var}(\bar{z}_{ON})$$

and eq. 8 follows.

Estimating the variance of the mean of an autocorrelated variable

In the variance reduction formula in eq. 9 we need to compute $\mathrm{var}(\bar{\varepsilon}_{ALL})$ and $\mathrm{var}(\bar{z}_{ON})$ for autocorrelated $\{z_i\}$ and $\{\varepsilon_i\}$. Generally, if the variables X_i, $i = 1,\ldots,n$, follow a first-order autoregressive process, the calculation of the variance of the mean is straightforward. If

$$X_i = aX_{i-1} + u_i$$

where the u_is are independent, zero mean, and identically distributed with $\mathrm{var}(u_i) = \sigma^2_u$, we have $\mathrm{var}(X_i) = \sigma^2_X = \sigma^2_u(1 - a^2)^{-1}$ and

$$(A4)\quad \mathrm{var}(\bar{X}) = \frac{\sigma^2_X}{n^2}\left\{n + 2\sum_{i=1}^{n-1} ia^{n-i}\right\}$$

(see, e.g., Brockwell and Davis 1996, chapters 2.3 and 2.4). In the special case when $a = 0$, it follows that $\mathrm{var}(\bar{X}) = \sigma^2_X/n$, and as $a \to 1$, we have $\mathrm{var}(\bar{X}) \to \sigma^2_X$, these two extremes having very different effects on the variance reduction.

Let $Y_i = X_{20i}$ for $i = 1,\ldots,n_{20}$, where $n_{20} = n/20$. In other words, the sequence $\{Y_i\}$ contains every 20th observation of $\{X_i\}$. Then we have

$$(A5)\quad \mathrm{var}(\bar{Y}) = \frac{\sigma^2_Y}{n^2_{20}}\left\{n_{20} + 2\sum_{i=1}^{n_{20}-1} ia^{20(n_{20}-i)}\right\}$$

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For higher-order autoregressive processes, the variance of the mean is more complicated to calculate analytically as a function of the autoregressive coefficients. It can be easily expressed (Brockwell and Davis 1996, p. 56) as a function of the autocovariances, but estimates of higher-order autocovariances are inaccurate. A better alternative is to use the autoregressive bootstrap as follows. Fit an autoregressive model to $\{X_i\}$ by estimating the autoregressive order and the autoregressive coefficients. Then, create m bootstrapped time series realizations $X_{1,k}^*,\ldots,X_{n,k}^*$, $k = 1,\ldots,m$, using the estimated autoregressive model. For each bootstrap realization, calculate the sample mean $\bar{X}_k^*$ and then estimate var($\bar{X}$) by $(m-1)^{-1}\sum_{k=1}^{m}(\bar{X}_k^* - \bar{X}^*)^2$, where $\bar{X}^* = m^{-1}\sum_{k=1}^{m}\bar{X}_k^*$.

If it is difficult to fit an autoregressive time series model to the data, then the block bootstrap (Politis et al. 1999) is an option assuming the time series to be essentially stationary (Politis et al. 1999, p. 101). There are several ways to block-bootstrap time series data. We have applied the method described in Politis et al. (1999, section 3.9). Given a set of observations, $X_1,\ldots,X_n$, generate a bootstrap replica by drawing with replacement n/L contiguous blocks of length L from $X_1,\ldots,X_n$. For example, with $n = 9$ and $L = 3$, the blocks may be X_7, X_8, X_9 and X_2, X_3, X_4 and X_6, X_7, X_8. Then, generate m bootstrap replicas, and for each bootstrap replica $\{X_k^*\}$, $k = 1,\ldots,m$, calculate the sample mean $\bar{X}_k^*$. Finally, estimate var($\bar{X}$) by $(m-1)^{-1}\sum_{k=1}^{m}(\bar{X}_k^* - \bar{X}^*)^2$.

Simulation of two time series with a given correlation and autocorrelation

In the simulation experiments, we need to generate time series having specific correlation and autocorrelation properties. To simulate two time series $\{\varepsilon_j\}$ and $\{z_j\}$ with a given correlation $\rho_{\varepsilon,z}$, autocorrelations $\rho_\varepsilon(1) = \rho_z(1) = a$ and variances σ_ε^2 and σ_z^2, we can use the model (again we refer to Brockwell and Davis 1996, chapters 2.1–2.4, for correlation properties of autoregressive and ARMA time series)

$$\varepsilon_j = a\varepsilon_{j-1} + u_j + w_j, \quad u_j \sim N(0, \sigma_u^2), \quad w_j \sim N(0, \sigma_w^2), \quad j = 1,\ldots,n_{\text{ALL}}$$

$$z_j = a z_{j-1} + v_j + w_j, \quad v_j \sim N(0, \sigma_v^2), \quad j = 1,\ldots,n_{\text{ALL}}$$

which gives a correlation

$$\text{(A6)} \quad \rho_{\varepsilon,z} = \text{corr}(\varepsilon_j, z_j) = \frac{(1-a^2)\sigma_{\varepsilon,z}}{\sqrt{(\sigma_u^2 + \sigma_w^2)(\sigma_v^2 + \sigma_w^2)}}$$

where $\sigma_{\varepsilon,z} = \text{Cov}(\varepsilon_j, z_j) = E(\varepsilon_j z_j)$. Simulated realizations are generated by using a Gaussian random number generator to generate time series $\{u_j\}$, $\{v_j\}$, and $\{w_j\}$. In order to do this, we need to specify σ_u^2, σ_v^2, and σ_w^2. Because $E(\varepsilon_j z_j) = E(\varepsilon_{j-1} z_{j-1})$, we have

$$E(\varepsilon_j z_j) = a^2 E(\varepsilon_j z_j) + \sigma_w^2$$

which means that

$$\text{(A7)} \quad \sigma_{\varepsilon,z} = \frac{\sigma_w^2}{1-a^2}$$

Putting eq. A7 into eq. A6 we get

$$\text{(A8)} \quad \rho_{\varepsilon,z} = \frac{\sigma_w^2}{\sqrt{(\sigma_u^2 + \sigma_w^2)(\sigma_v^2 + \sigma_w^2)}} = \frac{\sigma_w^2}{(1-a^2)\sigma_\varepsilon\sigma_z}$$

To generate time series with a given σ_ε^2, σ_z^2, and $\rho_{\varepsilon,z}$ under the restriction $\rho_{\varepsilon,z} < \min(\sigma_z/\sigma_\varepsilon, \sigma_\varepsilon/\sigma_z)$, choose

$\sigma_w^2 = \sigma_\varepsilon\sigma_z\rho_{\varepsilon,z}(1-a^2)$, $\sigma_u^2 = \sigma_\varepsilon^2(1-a^2) - \sigma_w^2$, and $\sigma_v^2 = \sigma_z^2(1-a^2) - \sigma_w^2$

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Is there a role for vision in the behaviour of sea lampreys (*Petromyzon marinus*) during their upstream spawning migration?

Thomas R. Binder and D. Gordon McDonald

Abstract: This study found no evidence to support a role for vision in the behaviour of sea lampreys (*Petromyzon marinus*) during their spawning migration. Blindness had no affect on the migratory propensity of lampreys, and control and blinded animals moved upstream at the same rate. The diel activity pattern of control and blinded lampreys was identical in both the laboratory and field. Activity was mostly nocturnal, but lampreys in the field did show some daytime activity. Diel activity broke down when lampreys were unable to locate suitable daytime refuge, but blindness did not affect the willingness or ability of lampreys to do so in either the laboratory or field. Laboratory studies indicate that light avoidance is mediated by dermal photoreceptors located in the tail, but the data suggests that refuge sites are sought out before sunrise using tactile and possibly hydraulic cues. Thus, light-induced searching only occurs when the sites chosen at night do not provide adequate concealment after sunrise.

Résumé : Notre étude ne trouve aucune indication qui appuie le rôle de la vision dans le comportement des grandes lamproies marines (*Petromyzon marinus*) durant leur migration de fraye. La cécité reste sans effet sur la propension à la migration chez les lamproies et les animaux témoins et aveuglés se déplacent vers l'amont à la même vitesse. Les patrons d'activité journalière des lamproies témoins et aveugles sont identiques, tant en laboratoire qu'en nature. L'activité est surtout nocturne, bien que les lamproies en nature soient partiellement actives le jour. L'activité diurne cesse lorsque les lamproies ne réussissent pas à trouver un refuge adéquat durant le jour, mais la cécité n'affecte ni la volonté, ni la capacité de demeurer en activité, tant au laboratoire qu'en nature. Nos études de laboratoire indiquent que l'évitement de la lumière se fait par l'intermédiaire de récepteurs dermiques situés dans la queue, mais nos données laissent croire que les lamproies recherchent les sites de refuge avant le lever du soleil à l'aide de signaux tactiles et peut-être hydrauliques. Ainsi, la recherche induite par la lumière n'a lieu que lorsque les sites choisis durant la nuit ne permettent pas une dissimulation suffisante après le lever du soleil.

[Traduit par la Rédaction]

Introduction

The upstream migration of sea lampreys (*Petromyzon marinus*) is, to a large extent, stimulated and guided by olfaction (Sorensen et al. 2005; Wagner et al. 2006), like salmon (Dittman and Quinn 1996; Døving and Stabell 2003). However, lampreys do not home to their natal stream (Bergstedt and Seelye 1995). Instead, sexually immature lampreys in the spawning phase are guided to suitable spawning streams by a multicomponent pheromone (migratory pheromone) released by stream-resident larval lampreys (Sorensen et al. 2005; Wagner et al. 2006). Once adults reach the spawning grounds and become sexually mature, they stop responding to the migratory pheromone (Bjerselius et al. 2000). The spermiating males build nests and release a second multicomponent bile acid pheromone (mating pheromone) to attract ovulating females (Li et al. 2002, 2003; Johnson et al. 2006). Thus, pheromones appear to have a primary role in guiding the spawning migration. However, there are two lines of evidence that suggest vision might also play a role. First, a 3:1 rod–cone ratio in the sea lamprey retina (Dickson and Graves 1979) suggests adaptation for night vision. Second, Purvis et al. (1985) demonstrated in upstream migrating lampreys that trapping efficiency was significantly improved in traps with lighted entrances.

Physiologically speaking, the sea lamprey eye is similar in structure to that of most vertebrates (Braun 1996). The retina contains two distinct photoreceptor types, which are homologous to the rods and cones found in other vertebrates (Govardovskii and Lychakov 1984; Collin et al. 1999), and the refractive properties of the optic lens suggest good visual resolution (Bantseev et al. 2005). During the parasitic phase, lampreys preferentially choose larger prey and display site fidelity in their attachments (Farmer and Beamish 1973; Farmer 1980). This suggests that vision is important in predatory behaviour. However, spawning phase sea lampreys cease feeding in the spring before beginning their migration

Received 1 November 2006. Accepted 28 May 2007. Published on the NRC Research Press Web site at cjfas.nrc.ca on 10 October 2007. J19630

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 doi:10.1139/F07-102 © 2007 NRC Canada

upstream to spawn (Bergstedt and Swink 1995). Therefore, the role of vision changes as lampreys enter into the spawning phase and change from predator to migrant.

The spawning migration is characterized by two distinct behaviours: (*i*) upstream migration at night and (*ii*) refuge-seeking and light avoidance during the day. However, near the end of the migratory season, lampreys lose their aversion to light and switch from strictly nocturnal to 24 h activity (Applegate 1950). Because this switch in activity is coincident with clouding-over of the dermal cornea of the eyes, it has been hypothesized that blindness disrupts normal refuge-seeking behaviour, thus leading to activity in the daytime (Applegate 1950; Manion and Hanson 1980). This conclusion, however, is complicated by the fact that light detection in lampreys is not limited to the eyes. The pineal gland of lampreys is photoreceptive, as are the dermal photoreceptors in the tail. The tail photoreceptors are part of the lateral line system. When illuminated, they cause burrowing in ammocoetes (larvae) and locomotor search activity in adults (Young 1935*a*; Ullén et al. 1993; Deliagina et al. 1995). In ammocoetes, where the eyes have not yet developed, the tail photoreceptors are integral to light avoidance. In the sighted adults, the response to tail illumination is undirected, but when the eyes are illuminated lampreys demonstrate negative phototaxis (Ullén et al. 1993).

The objective of this study was to determine what role, if any, vision plays in the behaviour of sea lampreys during their spawning migration. Lampreys were blinded by inserting an opaque rubber eye cap between the dermal and scleral cornea of each eye. The behaviour of control and blinded lampreys was compared using video and PIT (passive integrated transponder) tag telemetry in the laboratory and PIT tag and radio telemetry in the field. Laboratory studies addressed the following questions: (*i*) Is diel activity dependent on the ability of lampreys to find light refuge? (*ii*) Does blindness alter normal diel activity? (*iii*) What is the relative role of the eyes and the tail photoreceptors in refuge-seeking behaviour? The field study, carried out in a sea lamprey spawning stream, compared the migratory behaviour of blind and sighted lampreys and addressed the following questions: (*i*) Does blindness affect migratory propensity (tendency to move upstream)? (*ii*) Does blindness affect the rate of upstream movement? (*iii*) Does blindness alter diel activity and (or) refuge-seeking behaviour?

Materials and methods

Experimental animals

Source and maintenance

This study used 291 male and female spawning phase sea lampreys (192 in the laboratory and 99 in the field; sex ratio in lab and field ~1:1, mean length ± standard error (SE) = 49.4 ± 0.2 cm; mean mass ± SE = 254.3 ± 4.4 g). Lampreys used in laboratory studies were collected from the barrier traps on Duffins Creek (Ajax, Ontario) and the Humber River (Toronto, Ontario) during the first few weeks of the migration. They were transported to the Hagen Aqualab (University of Guelph, Ontario) and housed in temperature-controlled tanks (1.85 m diameter, total volume ~2000 L) under a 12 h photoperiod. Water in the holding tanks was maintained at 7 ± 1 °C, and fresh replacement water was supplied to the holding tank at a rate of 85 $L \cdot h^{-1}$. Holding the lampreys at low temperature halts migratory activity and delays their sexual maturation (Applegate 1950; Sjöberg 1977). Furthermore, the lower temperatures allowed us to keep animals healthy in the laboratory for almost 2 month longer than they would be in the field. All lampreys used in the laboratory studies were sexually immature. Lampreys in the field study were sexually immature at the time of release, but at least some matured by the end of the study period (as evidenced by spawning behaviour in the second week of the study). The procedures used in this study were approved by the Animal Care Committee at the University of Guelph and followed Canadian Council of Animal Care (CCAC) guidelines.

Insertion of PIT tags and radio telemetry transmitters

All lampreys were implanted with a PIT tag (Texas Instruments radio frequency identification; 23 mm, 0.65 g). In addition, 35 lampreys in the field experiment were also implanted with a radio telemetry tag (Holohil BD-2 transmitter; 16 mm, 1.20 g). Both tag types were implanted intraperitoneally under general anaesthesia (0.8 $mL \cdot L^{-1}$ of a 10% clove oil – ethanol mixture). An incision was made just off the ventral midline, approximately halfway along the length of the body. For PIT tag insertion, the incision was approximately 5 mm in length and was sealed with tissue adhesive (Vetbond™, 3M). For insertion of telemetry tags, the incision was approximately 10 mm in length and was closed using one or two sutures (Surgipro, 3-0 monofilament polypropylene). The telemetry tag antenna wire was threaded out through the body wall approximately 2 cm posterior to the incision.

Blinding procedure

Blinding was accomplished by inserting a 6–8 mm square of sterilized black opaque balloon rubber (eye cap) into the capillary space between the dermal and scleral cornea of each eye. This technique was used over traditional procedures (i.e., cauterization or optic nerve transection) because of its low level of invasiveness. This allowed for minimal recovery time, especially in the field study where setting up long-term holding facilities was problematic. The decision to use balloon rubber was based on its flexibility. Its ability to block all incoming light was confirmed with a photometer and a 2000 lux light source. Each eye cap was inserted through a 2–3 mm incision made along the posterior edge of the dermal cornea. Once inserted, the eye cap was flattened out and centered over the pupil. In this configuration, the eye cap covered the entire eye and acted like an opaque contact lens. Vetbond™ was applied to seal the incision.

In the laboratory, blinded lampreys were recovered for at least 36 h before being used in the light-avoidance experiments and at least 5 days before being used in spontaneous activity experiments. In the field, lampreys were blinded during tagging and then were recovered for 1 h before release. With the exception of insertion of eye caps, control and blinded lampreys in all experiments were treated identically. No sham eye treatment was used because the incision often caused the dermal cornea to cloud over. Blinding did not affect the overall health of the animals, although minor

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infection was noted in the eyes of some lampreys (less than 10%). Each time lampreys were handled, their eyes were checked to ensure that the eye caps remained in place. No eye caps were lost during the study.

Experimental series

Spontaneous locomotor activity

Spontaneous locomotor activity was measured in a 1.85 m diameter annular raceway (Fig. 1*a*; channel width ~60 cm, water depth ~30 cm), fitted with four PIT tag antennae loops. The antennae were oriented at 90° to one another. Each time a lamprey passed through an antenna, the datalogger (Texas instruments series 2000 multiplexed reader unit and Palm™ datalogger, Oregon RFID) recorded the time of detection, antenna number (1–4), and PIT tag number. These records were then used to quantify activity in the tank (movement from one antenna to another = one movement). Water was circulated in the tank to produce a slow counterclockwise current (~20 cm·s^{-1}, ~0.4 body lengths (BL)·s^{-1}), and fresh replacement water was supplied to the tank at a rate of 75 L·h^{-1} (total volume of the system ~900 L). The tank had a lightproof hood that was programmed to provide a 12 h photoperiod (0700 to 1900). The light (~50 lux at water surface) brightened and dimmed gradually over 30 min to simulate dawn and dusk. Three polyvinyl chloride (PVC) platforms (40 cm × 50 cm, cable-tied to two halves of a 15 cm diameter PVC pipe) were distributed within the tank (Fig. 1*a*) to provided refuge sites for the lampreys (total refuge area = 0.6 m^2, approximately 25% of the raceway floor).

Activity was monitored at 15 ± 1 °C under a 12 h photoperiod. Lampreys were first temperature-acclimated before being transferred into the experimental apparatus. Temperature in the holding tank was raised from 7 to 15 °C over 8 h, and the lampreys were given 16 h to acclimate to the new temperature before being transferred to the raceway. They were allowed an additional 6 h to settle in the raceway before activity recording began. Three 48 h trials were run. In two of the trials, the activity pattern of control (N = 9 in each trial) and blinded lampreys (N = 9 in each trial) were compared with refuge sites available. The data from the two trials were pooled for analysis (total of 36 individuals). In the third trial (N = 20 controls), the refuge platforms were removed to assess whether the normal diel activity pattern displayed by lampreys is dependent on their ability to find light refuge. The activity of each individual was quantified from the PIT tag detections.

Statistical comparisons were done using nonparametric statistics because activity in the raceway was not normally distributed. Reproducibility of individual activity in the annular raceway was tested in both control and blinded lampreys by comparing the total number of movements of individuals on the first and second day of the 48 h trial (Spearman's rank correlation, α = 0.05; JMP v. 4.0, SAS Inc., Cary, North Carolina). The total activity (sum of all movements during the study) of control and blinded lampreys were compared using a Wilcoxon rank-sum test (JMP v. 4.0; α = 0.05), and Wilcoxon paired-sample tests (Zar 1999, pp. 165–167; α = 0.05) were used to compare the number of upstream (against flow) and downstream (with flow) movements (paired by individual) within each of the two groups.

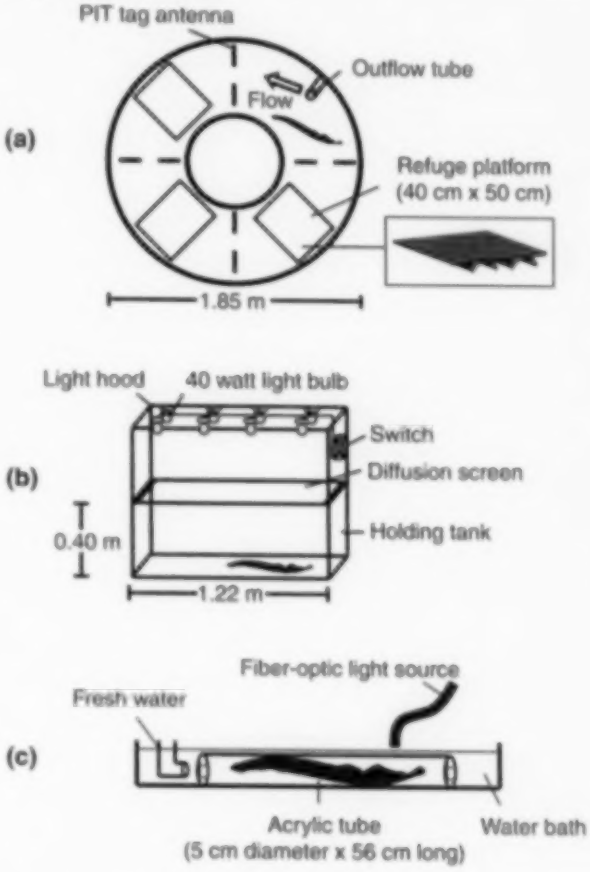

Fig. 1. Schematic diagrams of apparatuses used in laboratory studies. (*a*) Annular raceway for monitoring spontaneous activity. Water was circulated in the tank to produce a counterclockwise current of ~20 cm·s^{-1}. Four passive integrated transponder (PIT) tag antennas monitored activity, and three polyvinyl chloride (PVC) platforms provided refuge sites for the sea lampreys (*Petromyzon marinus*). (*b*) Light chamber for recording the response to whole body illumination (~1160 lux). Response was recorded with four underwater infrared cameras. (*c*) Setup for directed illumination study. Lampreys were confined within a clear acrylic tube and submerged in a water bath. A fiber-optic light source was used to direct light (~1050 lux) at either the head or tail. The response was recorded with an infrared camera, suspended above the tube.

Refuge-seeking behaviour

Whole body illumination

A light chamber (Fig. 1*b*) was used to simultaneously illuminate the whole body of the lamprey. The light-tight chamber consisted of a holding tank (120 cm × 40 cm × 40 cm) and light hood. The inside walls and floor of the tank were black, and the inside of the light hood was white. A white translucent diffusion screen separated the tank from the light

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hood. Water depth was maintained at 20 cm (water volume ~100 L), and replacement water (14 ± 1 °C) was supplied at approximately 2 L·min^{-1}. The light hood contained eight 40 W incandescent light bulbs. When lights were turned on, light intensity at the surface of the water was ~1160 lux, similar to that experienced on a cloudy day. Activity in the tank was monitored using four underwater cameras (black and white charge-coupled device (CCD) camera with infrared emitters, model No. KG-190; CCTV-Wholesalers, New Orleans, Louisiana). The video feeds were multiplexed with a quad multiplexer (CCTV-Wholesalers) so that all four camera feeds were visible on the monitor at the same time. The resulting image was recorded with a VCR.

Forty-two lampreys (21 control, 21 blinded) were placed individually into the light chamber and were dark-acclimated for at least 10 min. At the end of acclimation, the lampreys were required to remain motionless for at least 1 min before the lights were turned on. Response latency (time from lights on to locomotor response) was calculated for each individual from the video recording. Time-to-event analysis (univariate survival analysis in JMP v. 4.0) was performed using the Kaplan–Meier method to compare the response of control and blinded individuals. Significance was assigned using a log-rank test ($\alpha = 0.05$). This procedure replaces the Wilcoxon rank-sum test (for two groups) or Kruskal–Wallis test (for more than two groups) in situations where the data set is censored (Hosmer and Lemeshow 1999). In this study, response latencies could not be assigned to lampreys that failed to respond within the allotted 60 s. Those data were therefore treated as censored data points in the analysis (i.e., response latency was >60 s).

To compare the search behaviour of control and blinded lampreys, the experiment was repeated using a center partition to create a dark chamber, which the lampreys (20 control and 20 blinded) could enter to take refuge from the light. A 5 cm diameter hole in the center of the partition allowed passage from one chamber to the other. The lights over one chamber were illuminated while the other chamber remained dark. The hole was plugged with a stopper until the lights were turned on to prevent lampreys from moving between chambers during the acclimation period. The stopper was then pulled from the hole using a string that led outside the chamber. The search behaviour of control and blinded lampreys was determined from the video record. Wilcoxon rank-sum tests (JMP v. 4.0; $\alpha = 0.05$) were used to compare (*i*) the time for control and blinded lampreys to pass into the dark chamber and (*ii*) the rate at which control and blinded lampreys moved from one corner to the next while searching the chamber for refuge (quantitative measure of search activity).

Directed illumination

The directed illumination apparatus (Fig. 1*c*) consisted of a clear acrylic tube (5 cm diameter × 56 cm long) and a fiber-optic light source (Schott Mainz, model KL 150B; 15 V – 150 W quartz lamp). The lampreys were confined within the apparatus by plastic mesh, which covered both ends of the tube. The tube was submerged in a water bath (14 ± 1 °C), and fresh water was directed through the tube. The light source illuminated an area approximately 7 cm in diameter at an intensity of ~1050 lux. A camera suspended above the tube captured the response of the lampreys, and the video signal was recorded with a VCR.

Lampreys were placed individually into the acrylic tube of the directed illumination apparatus and were acclimated for at least 10 min in complete darkness. At the end of acclimation, the light source was manually positioned to illuminate either the head or tail of the lamprey. Dim red light was used to enable the experimenter to see where the light source was pointing (lampreys showed no reaction to the red light). Control ($N = 18$) and blinded ($N = 18$) lampreys were tested for a response to light directed at the head, and control lampreys ($N = 18$) were tested for a response to light directed at the tail. When directed at the head, the light was pointed at either the left or right eye (incident angle was ~65°). When illuminating the tail, the light was pointed at the posterior region of the tail, centered approximately 6 cm from the tip (incident angle was ~45°). The lampreys were given 60 s to respond to the light, and response latency was determined for each individual from the video record. Time-to-event analysis (Kaplan–Meier method with log-rank test, JMP v. 4.0, $\alpha = 0.05$) was used to compare latency among the three treatment groups as well as with those obtained in the whole body illumination experiment.

Role of vision in the field

The migratory behaviour of control and blinded lampreys was monitored in Duffins Creek (Ajax, Ontario; Fig. 2) during a 2-week period in the last half of the migratory season (26 May – 9 June 2005). During this period, daytime high temperatures in the stream ranged from 17 to 27 °C and nighttime lows from 13 to 20 °C. Lampreys were captured in the sea lamprey barrier trap, tagged, and then released in eight separate groups between 26 May and 3 June. Half of the lampreys released in each group were blinded before being released into the stream. Only lampreys with clear, healthy-looking eyes were used in the experiment. In total, 99 lampreys were fitted with PIT tags. Of those, 35 also received a telemetry tag. Lampreys were released between 1130 and 1330, except on 26 May when they were released at 1930. This was because the trap operators were delayed by several hours owing to unusually high lamprey numbers in both the Humber River and Duffins Creek sea lamprey traps. Lampreys with telemetry tags were released ~3.6 km downstream of the sea lamprey barrier, while those with PIT tags alone were released ~640 m downstream of the barrier (Fig. 2).

A stationary PIT tag antenna (single-loop, 8-gauge, oxygen-free wire, total length = 30 m, maximum depth = 0.65 m) spanned the width of the stream approximately 10 m downstream of the sea lamprey barrier. This antenna monitored movement patterns downstream of the barrier and provided an estimate of migration rate (time from release point to antenna).

The telemetry tags were located each afternoon (between 1300 and 1700), using a Lotek SRX-600 receiver and three-element Yagi antenna (AF Antronics, model F150-3FB 07645). The detection range of the receiver varied depending on the location, but averaged between 100 and 200 m. The tracking team moved downstream by canoe from the sea lamprey barrier to the mouth of the stream (~6 km). When a

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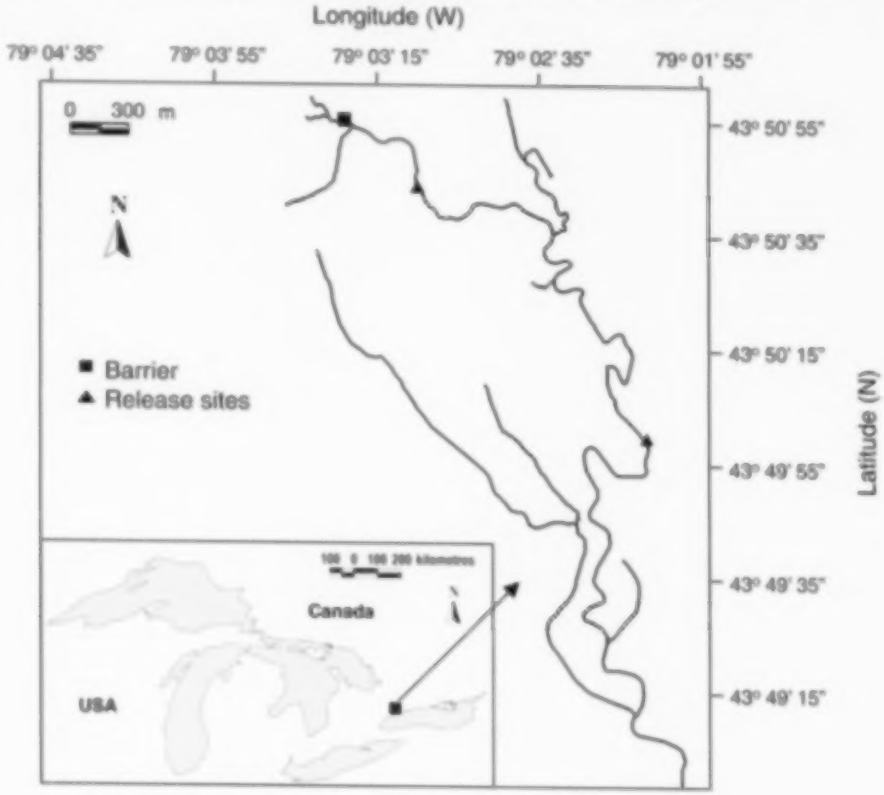
Fig. 2. Duffins Creek (Ajax, Ontario) field study site, located on the western shore of Lake Ontario. Inset shows position within the Great Lakes region. Sea lampreys (*Petromyzon marinus*) were released either 640 m or 3.6 km downstream (triangles on the map) of the sea lamprey barrier (square on the map) between 26 May and 9 June 2005. Behaviour was monitored by mobile radio telemetry and by an instream passive integrated transponder (PIT) tag antenna located ~10 m downstream of the barrier.



signal was detected, the team got out of the canoe and homed in on the precise location of the lamprey by scanning with the antenna while decreasing the receiver gain. The position of the lamprey within the stream (bank or midstream) and the type of refuge being used (e.g., rocks, overhanging brush) were recorded.

Data from lampreys released 640 m and 3.6 km downstream were analyzed separately. Fisher's exact tests were used to compare the proportion of lampreys from each treatment group that returned to the barrier and the proportion of individuals that demonstrated daytime activity, based on PIT tag detections (JMP v. 4.0; $\alpha = 0.05$). For lampreys released 640 m downstream, return times (time from release to first detection at the PIT tag antenna) were log-transformed to fit a normal distribution (Shapiro–Wilk test; $\alpha = 0.05$; JMP v. 4.0). A randomized block analysis of variance (ANOVA, $\alpha = 0.05$; Minitab, Minitab Inc., Penn State University, Pennsylvania) was then used to compare return time in control and blinded individuals, blocked by release group (four release groups). Return time was not compared statistically in telemetered lampreys (released 3.6 km downstream), because the telemetry tags interfered with the ability of the PIT tag antenna to read the PIT tags.

Results

Spontaneous locomotor activity

Locomotor activity was almost exclusively nocturnal when daytime refuge sites were available (Figs. 3*b* and 3*c*). Activity peaked 2–3 h after dark and then gradually declined to low levels by 0400. Most individuals stopped moving at least 3 h before dawn, but a few remained active into the first hour of daylight (Figs. 3*b* and 3*c*). This was associated with seeking refuge because removing refuge sites resulted in a substantial increase in daytime activity in almost all individuals, especially in the first 4 h following dawn (Fig. 3*a*). Moreover, the inability to locate daytime refuge sites destabilized the normal nocturnal rhythm. Far fewer lampreys were active on the second night of the trial, which resulted in a 64% decrease in total activity between the first and second dark periods (Fig. 3*a*).

There was no difference in the pattern or amount of activity displayed by control and blinded lampreys (Figs. 3*b* and 3*c*). Individual activity was highly variable within both groups, but was repeatable over the 2-day trial (Spearman's rank correlation; control: $r = 0.519$, $P = 0.027$; blinded: $r = 0.672$, $P = 0.002$). Moreover, there was no significant differ-

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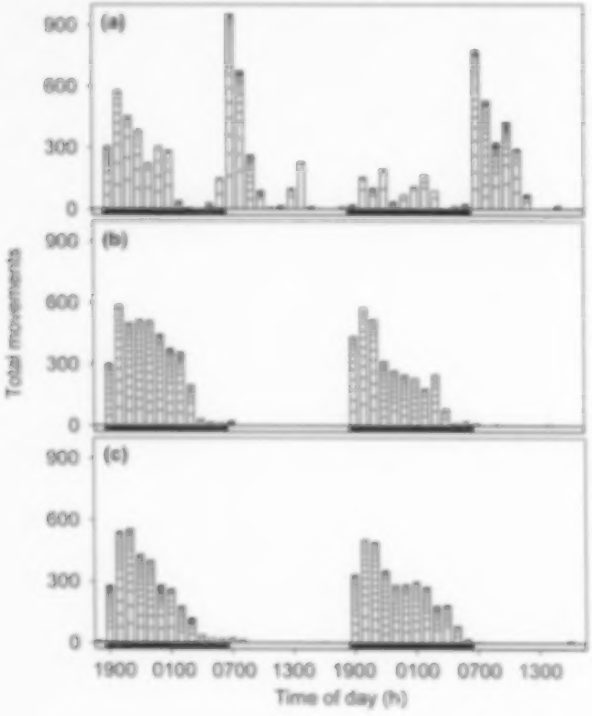
Fig. 3. Activity pattern of control and blinded sea lampreys (*Petromyzon marinus*) in an annular raceway with and without refuge sites available. (*a*) Controls ($N = 20$) without daytime refuge. (*b*) Controls ($N = 18$) with daytime refuge. (*c*) Blinded ($N = 18$) with refuge. Stacked bars show individual contributions to total activity in each 1 h interval. Panels (*b*) and (*c*) present results pooled from two separate trials. Light and dark horizontal bars along the *x* axis identify day and night, respectively.



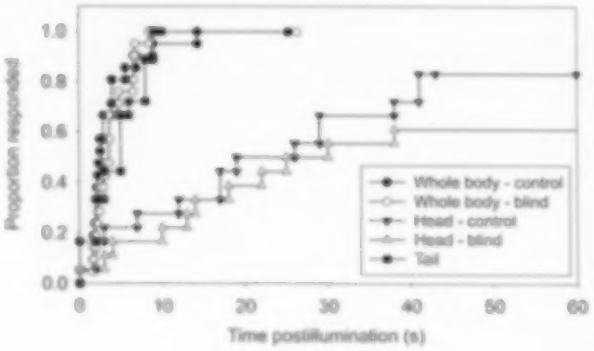
ence in total activity between control and blinded animals (Wilcoxon rank-sum test, $Z = 0.554$, $P = 0.580$). The median number of movements during the 48 h trial was 340 movements for controls (range: 9–866 movements) and 250 movements for blinded lampreys (range: 61–1042 movements). In both groups, daytime activity represented less than 4% of their total activity, and almost all of this activity occurred within 2 h of dawn. In addition, both control and blinded lampreys demonstrated a significant bias towards movements in the downstream direction (Wilcoxon paired-sample test; control: $T = 155.5$, $P < 0.005$; blinded: $T = 160.5$, $P < 0.001$). The ratio of upstream to downstream movements was approximately 1:1.25 in both groups.

Refuge-seeking behaviour

Whole body illumination

During dark acclimation, lampreys briefly explored the apparatus (<5 min) and then came to rest and attached to the side or bottom of the chamber (Fig. 1*b*). The response to illumination consisted of tail deflections followed by detachment and swimming movements. All lampreys, whether blinded or controls, responded to whole body illumination within 30 s. Response latencies were nearly identical in control and blinded lampreys (time-to-event analysis: log-rank test, $\chi^2 = 1.435$, $P = 0.694$; Fig. 4). Latency ranged from 1.6 to 26.3 s in controls (median = 2.5 s) and from 1.6 to 25.2 s in blinded lampreys (median = 3.6 s).

Fig. 4. Kaplan–Meier time-to-event curves comparing response latency following whole body and directed illumination. The response to head illumination in control ($N = 18$) and blinded ($N = 18$) sea lampreys (*Petromyzon marinus*) was significantly slower than that in the other three groups (time-to-event analysis, $P < 0.050$). Response latency was not significantly different among tail-illuminated ($N = 18$) and whole-body-illuminated control ($N = 21$) and blinded ($N = 21$) lampreys (time-to-event analysis, $P > 0.050$).



The subsequent light-induced search pattern of blinded lampreys was not noticeably different from controls. Lampreys mainly probed the periphery of chamber, particularly in the corners. Generally, the lampreys moved between adjacent corners, following either a clockwise or counterclockwise path. Diagonal movements across the chamber were rare, but the lampreys sometimes changed direction several times.

In the two-chambered experiment, most lampreys continually probed the lighted chamber until they passed through the partition and then quickly came to rest in the dark. All lampreys entered the dark chamber within 6 min (range: 0.23 to 6.57 min for controls and 0.02–2.87 min for blinded lampreys). The median time for controls was longer than that of their blinded counterparts (1.78 min in controls vs. 1.24 min in blinded), but the difference was not significant (Wilcoxon rank-sum test, $Z = 1.785$, $P = 0.074$). The rate at which lampreys moved from one corner to another was used as a quantitative measure of search activity. The two groups searched for refuge with equal intensity, making about 10 (median = 10.4 in controls and 10.1 in blinded) corner changes per minute (Wilcoxon rank-sum test, $Z = 0.501$, $P = 0.617$).

Directed illumination

Lampreys came to rest in the clear acrylic tube (Fig. 1*c*), but not all attached. The response to head illumination was not significantly different in control and blinded lampreys (Fig. 4; time-to-event analysis: log-rank test, $\chi^2 = 1.435$, $P = 0.231$). Illumination of the head alone elicited locomotion in 15 of 18 controls and in 11 of 18 blinded lampreys. The median response latency was 27.5 s in controls and 34.0 s in blinded lampreys. Thus, the response to head illumination was approximately sixfold slower than that evoked by whole

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Table. 1. Summary of migratory and refuge behaviours of 99 sea lampreys (*Petromyzon marinus*) tagged and released either 640 m (passive integrated transponder (PIT)-tagged only) or 3.6 km (radio- and PIT-tagged) downstream of a sea lamprey barrier between 26 May and 9 June 2005 (Duffins Creek, Ajax, Ontario).

	Released 640 m downstream		Released 3.6 km downstream	
	Control	Blinded	Control	Blinded
Migratory behaviour				
No. released	32	32	17	18
No. returned to barrier	31	30	14	13
No. not located after release	1	2	1	2
Mean (±SE) time to barrier	23.2 (±2.8) h	28.8 (±7.5) h*	2.21 (±0.45) nights†	2.15 (±0.40) nights†
Shortest time to barrier (h)	0.87	0.86	NA‡	NA‡
Refuge position (no. of observations)				
Refuge (midstream)	NA‡	NA‡	27	21
Refuge (along bank)	NA‡	NA‡	35	32
Along the face of barrier	NA‡	NA‡	8	5
Unconcealed in stream	NA‡	NA‡	7	3
Refuge type (no. of observations)				
Overhanging bank	NA‡	NA‡	23	15
Under or among large rocks	NA‡	NA‡	15	22
Among fallen brush	NA‡	NA‡	15	12
Within deep pools	NA‡	NA‡	6	4
In mud along bank	NA‡	NA‡	3	0

*Mean skewed by one individual that took 8 days to return to the barrier (outlier excluded: mean = 23.0 ± 5.0 h).
†Migration rates based on PIT tag antenna, telemetry, and trap data, because telemetry tags interfered with the PIT tag antenna's ability to detect PIT tags (no precise rate could be determined).
‡Not applicable.

body illumination (~30 vs. ~5 s) (Fig. 4; time-to-event analysis: log-rank test, $\chi^2 = 52.930$, $P < 0.001$).

In contrast, tail illumination produced a locomotor response in all individuals, and the response latency was not significantly different from that elicited by whole body illumination (Fig. 4; time-to-event analysis: log-rank test, $\chi^2 = 2.337$, $P = 0.306$). The median response latency for tail illumination was 6 s, and all lampreys tested responded within 10 s.

Role of vision in the field

Blindness had no obvious effect on the migratory behaviour of lampreys (Table 1). There was no significant difference between the fraction of control and blinded lampreys that returned to the barrier after release (Table 1; Fisher's exact test, $P > 0.999$ for 640 m and $P = 0.691$ for 3.6 km) — 92% of controls and 86% of blinded lampreys resumed their upstream migration. In total, 11 lampreys (four control and seven blinded) did not return to the barrier. Only three (one control and two blinded) of 64 lampreys released 640 m downstream did not return to the barrier, while eight (three control and five blinded) of 35 lampreys released 3.6 km downstream did not return. Three of the eight lampreys that did not return from 3.6 km downstream disappeared from the main stream within 24 h of release. Their fate is unknown.

The migration rates (time to reach the barrier) of control and blinded lampreys released 640 m downstream of the barrier were also not significantly different (randomized block ANOVA, $F = 0.02$, $P = 0.896$). Most lampreys returned to the barrier within 2 nights. The mean return time for controls was 5 h shorter than that for blinded individuals, but this difference was attributable to one blind individual that took 8 days to return to the barrier. With this outlier removed, the mean return time of blinded lampreys was identical to that of controls.

Precise return times could not be determined for lampreys released 3.6 km downstream because the radio telemetry tags interfered with detection of the PIT tags. The PIT tag antenna failed to detect 5 of the 12 telemetered lampreys that were caught in the barrier trap. This was not an issue in lampreys that had only a PIT tag; the antenna detected all 14 lampreys recaptured from this group. Taken together the PIT tag antenna and recapture data showed that almost all of the lampreys that returned to the barrier from 3.6 km downstream (12 of 14 control, 11 of 13 blinded) did so within 3 nights.

Blinding did not diminish the willingness or ability of lampreys to find refuge sites. Few lampreys were found in the open during the tracking period, and the refuge sites used by control and blinded lampreys were not noticeably different (Table 1). Neither group showed a preference for bank or midstream refuge. Lampreys in both groups were most commonly found under the concrete ledge on either side of the barrier, under and around large rocks or fallen brush, or in deep pools.

Furthermore, the daily pattern of migratory activity did not differ between control and blinded lampreys (Fig. 5). The majority of activity in the two groups occurred at night, but some daytime activity (0700 to 1900) was recorded in both groups on days 4 through 11 of the 2-week study. Daytime activity was confined to the afternoon, peaking between 1500 and 1600. Control lampreys were slightly more active than their blinded counterparts between 0000 and 0600.

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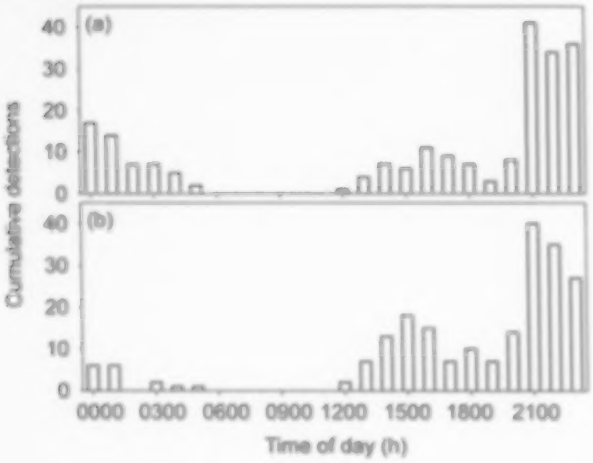
Fig. 5. Timing of migratory activity in (*a*) control ($N = 42$) and (*b*) blinded ($N = 40$) sea lampreys (*Petromyzon marinus*) in the field. Lampreys were released into the stream in eight separate groups between 26 May and 3 June. Half of each group was blinded. Activity was monitored for 2 weeks (26 May – 9 June). Graphs display cumulative detections (219 for controls and 211 for blinded), measured by a stationary passive integrated transponder (PIT) tag antenna located ~10 m downstream of a sea lamprey barrier.



However, activity in both groups was highest between 2100 and 2400. Activity during this period accounted for approximately 50% of the total activity for both groups. Moreover, the proportion of control (20 of 43) and blinded (23 of 40) lampreys that were active during the day was not significantly different (Fisher's exact test, $P = 0.382$).

Discussion

This study used both laboratory and field experiments to investigate the role of vision in the behaviour of upstream-migrating sea lampreys. This approach allowed us to conduct highly controlled laboratory experiments, while ensuring that the results obtained in the laboratory were consistent with behaviour in the field. Overall, we found no evidence to support a role for vision in the behaviour of sea lampreys during upstream migration.

Neither the migratory propensity nor the rate of upstream movement of sea lampreys in the field was affected by blindness. These findings provide further support for olfactory-based navigation in sea lampreys. In fact, both control and blinded lampreys in the present study displayed negative rheotaxis in the circular raceway, where there was no larval pheromone. This result is consistent with field observations made by Johnson et al. (2006), who noted that ovulating females made 16 times more downstream movements when washings from males (mating pheromone) were pulsed rather than continuously applied. Thus, the weight of evidence suggests that the sea lamprey migration fits the salmon olfactory-induced rheotaxis model of migration (Døving and Stabell 2003). Furthermore, recent evidence that European river lampreys (*Lampetra fluviatilis*) are also attracted to a larval pheromone (Gaudron and Lucas 2006) suggests that this type of navigation might be common across the various lamprey taxa.

Nonetheless, to make upstream progress, fish must swim at a velocity that is higher than that of the surrounding water and must therefore have a mechanism for determining water velocity. The lateral line of fishes is used to gauge water velocity, but requires either a visual or tactile fixed frame of reference (Montgomery et al. 1997). The fact that control and blinded lampreys swam upstream at the same rate indicates that lampreys do not use a visual fixed frame of reference to determine velocity. Instead, lampreys periodically attach to the substrate using their oral disk. It has been suggested that the purpose of this behaviour is to allow recovery from periods of strenuous exercise (Quintella et al. 2004). However, attachments, especially those of short duration, probably also provide an important frame of reference for mapping the local hydraulics, including water velocity.

Blindness had no affect on diel activity in either the laboratory or field, but both groups displayed some daytime activity in the field. This indicates that the switch to 24 h activity at the end of the season is not due to blindness, as has been previously suggested (Applegate 1950; Manion and Hanson 1980). Sjöberg (1977) noted that the switch to 24 h activity in the European river lamprey was delayed by about a month by holding them in cold water for 6 weeks before allowing them to progress through the normal rise in temperature that occurs during the spawning migration. Thus, the switch could be triggered by temperature. However, because sexual development is also delayed by holding in cold water, an alternate explanation could be that the switch is triggered by sexual maturation and the onset of spawning behaviour. In the current study, daytime temperatures in the field increased from 17 to 27 °C over the study period. Moreover, recent laboratory studies have shown that sexually immature lampreys become active during the day when they are held at temperatures above 20 °C (T.R. Binder and D.G. McDonald, unpublished data). While this finding indicates a role for temperature in the switch from nocturnal to 24 h activity, the possibility that sexual maturation also causes lampreys to become active during the day cannot be disregarded. To address this issue, future research should focus on determining the affect of temperature on activity in sexually mature individuals.

The light–dark cycle plays a crucial role in shaping the activity of sea lampreys. Normal diel activity in the lampreys was dependent on their ability to locate light refuge during the day. In the laboratory, lampreys became active during the daytime when they were unable to find refuge. However, when refuge sites were available, the activity pattern of control and blinded lampreys was the same. Our results indicate that light avoidance, as it relates to refuge behaviour, is not mediated by the eyes. Control and blinded lampreys showed an identical response to light directed at the head, suggesting that the pineal gland may mediate the response to head illumination. However, Young (1935*b*) noted in two species of lampreys (*L. fluviatilis* and *Lampetra planeri*) that the head remained sensitive to light even after the eyes and pineal gland were removed. Thus, other photoreceptors in the head may contribute to the response. Nonetheless, the response to head illumination was slower and less consistent than the whole body response, indicating

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that the receptors that are primarily responsible for light avoidance are not located on the head. Indeed, it was the locomotor response to tail illumination that mirrored the whole body response in both control and blinded lampreys. This indicates that light avoidance in adult lampreys is primarily mediated by the tail photoreceptors, as it is in ammocoetes (Young 1935*a*).

When exposed to light, the lampreys probed along the walls of the chamber in search of some escape from the light. The two groups deployed identical search strategies, and blinded lampreys found light refuge as quickly as sighted lampreys in both the spontaneous activity tank and light chamber. Moreover, the refuge sites chosen by blinded lampreys in the field were not noticeably different than those used by controls. This indicates that vision plays little role and suggests that lampreys primarily use tactile and (or) hydraulic cues to search for refuge. This observation is not surprising, given that most of the migratory activity of lampreys typically ceases before sunrise (Hardisty and Potter 1971; Kelso and Gardner 2000; Almeida et al. 2002). Thus, lampreys probably locate refuge sites in the dark when vision is limited. Our activity data supports this conclusion. In the "without refuge" trial, lampreys exposed to light showed an immediate increase in activity. When refuge sites were available, most lampreys stopped moving at least 3 h before dawn and only a few were active after the light was turned on. This indicates that the majority of lampreys found refuge in the dark and consequently were not exposed when the light in the tank turned on. In this context, light-induced refuge-seeking would only occur when refuge sites chosen in the dark do not provide the lampreys with adequate cover during the day.

In conclusion, this study found no evidence of a role for vision in the behaviour of upstream-migrating sea lampreys. The relative unimportance of vision during the spawning migration is probably related to the nocturnal nature of the lampreys and seems to reflect a large dependency on olfactory signals. However, this study did not test the effect of blindness on nest construction and spawning behaviour, which is well orchestrated and often occurs during the day (Applegate 1950). Thus, further work is required to determine whether blindness has an effect on the reproductive success of individuals. Furthermore, given the findings of this study and the fact lampreys are negatively phototaxic, additional research is needed to explain the observation by Purvis et al. (1985) that substantially more lampreys were caught in a trap when the entrance was lit compared with when it was not.

Acknowledgements

We thank Stacey Lee-Jenkins, Marcie Ninness, and Emily Smith for their assistance, both in the laboratory and in the field. Thanks also go to Bob Frank, Matt Cornish, and Micah Herriot for helping to set up and maintain our laboratory facilities in the Hagen Aqualab, University of Guelph. Special thanks go to Dr. Rob McLaughlin and Dr. Don Stevens for their helpful comments and to Dr. Chris Bunt for his help with the radio telemetry and the development of the blinding procedure. Finally, we thank the two anonymous referees for their helpful comments and suggestions on the manuscript. Funding for this research was provided by the Great Lakes Fishery Commission.

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Effects of colonizing predators on yellow perch (*Perca flavescens*) populations in lakes recovering from acidification and metal stress

Kelly A. Lippert, John M. Gunn, and George E. Morgan

Abstract: We examined the effects of predator species on yellow perch (*Perca flavescens*) from lakes in the Sudbury mining region (Ontario, Canada), where fish communities are just beginning to recover from the effects of decades of acidification and metal stress. The predation regimes investigated included six lakes with no predators, four lakes recently invaded (≤3 years) by predators, and eight lakes with well-established (≥15 years) predator populations. Netting survey results indicated that yellow perch biomass was 69% lower in lakes with predators. In lakes recently invaded by predators, perch displayed earlier ontogenetic diet shifts from zooplankton to benthos and much poorer growth during their first growing season. Energy investment (lipid content per egg) in eggs by mature females was also less in the lakes recently invaded by predators. Growth potential in adult perch (age 1), estimated by nucleoside diphospholkinase, was highest in lakes with established predators. Body condition, controlling for size of perch, was also greatest in established predator lakes. Perch body shape in lakes with predators showed a shift from a typical streamlined, pelagic body form towards a deeper-bodied benthic body form, a change that may increase foraging efficiency for benthos or represent an antipredator strategy.

Résumé : Nous examinons l'effet des espèces prédatrices sur la perchaude (*Perca flavescens*) dans des lacs de la région minière de Sudbury (Ontario, Canada) où les communautés de poissons commencent tout juste à se remettre après des décennies d'acidification et de stress dû aux métaux. Les régimes de prédation étudiés comprennent six lacs sans prédateur, quatre lacs envahis récemment (≤3 ans) par les prédateurs et 15 lacs avec des populations de prédateurs bien établies (≥15 ans). Les résultats d'un échantillonnage au filet indiquent que la biomasse des perchaudes est de 69 % inférieure dans les lacs avec prédateurs. Dans les lacs récemment envahis par les prédateurs, les perchaudes devancent leur passage ontologique d'une diète de zooplancton à une de benthos et leur croissance durant leur première saison de développement est de beaucoup inférieure. L'investissement d'énergie dans les oeufs (contenu lipidique de l'oeuf) par les femelles matures est aussi inférieur dans les lacs récemment envahis par les prédateurs. Le potentiel de croissance chez les perchaudes adultes (âge 1), estimé par la nucléoside diphosphokinase, est maximale dans les lacs avec des prédateurs établis. La condition corporelle des perchaudes, après correction pour la taille, est aussi maximale dans les lacs avec des prédateurs établis. La forme du corps des perchaudes dans les lacs avec prédateurs change d'un aspect fuselé typique d'un corps pélagique à une forme benthique à hauteur du corps plus marquée, un changement qui peut augmenter l'efficacité de la recherche de nourriture benthique ou alors représenter une stratégie anti-prédateur.

[Traduit par la Rédaction]

Introduction

Ecologists have long recognized that predators are among the most important factors structuring aquatic communities (Reznick et al. 1990). Much of the early literature focused on the direct lethal effect of predators, but the mere presence of a predator is now widely known to exert profound indirect effects on entire prey communities (Lima 1998). Changes in the behavior of the prey to avoid detection, encounter, and capture by the predator are common indirect effects that often involve major changes in habitat use (Lima and Dill 1990). Such changes in habitat use can be quite costly to some prey species if they are forced to confine themselves to refuge areas where they initially, or eventually (because of their numbers), lack abundant or high quality food (Werner and Gilliam 1984). These indirect density-dependent effects of predation can also influence the phenotypic expression of prey communities leading to changes in survival, growth, reproductive investment, physiology, and morphology (Werner et al. 1983; Werner 1984; Fraser and Gilliam 1992). The morphology changes (i.e., changes in body form), like the habitat changes, may help reduce predation pressure by reducing encounters with predators or by allowing prey to escape after an encounter. Such morphological changes can be stimulated by visual or olfactory cues from predators (Brönmark and Miner 1992; Holopainen et al. 1997), and like many aspects of the predation effects, the morphological changes among individuals can occur very rapidly.

Yellow perch (*Perca flavescens*) and its close relative the Eurasian perch (*Perca fluviatilis*) are widely distributed in

Received 30 September 2006. Accepted 24 May 2007. Published on the NRC Research Press Web site at cjfas.nrc.ca on 10 October 2007.
J19570

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 doi:10.1139/F07-097 © 2007 NRC Canada

the northern hemisphere and represent prey species that coexist with many different predators. However, few studies have attempted to assess the effects of predators on a multitude of life history characteristics of perch. Controlled pond experiments have shown that small perch, vulnerable to predation, moved from open-water habitats to littoral refuges to avoid predators (Werner et al. 1983). Also, vulnerable size classes (<120 mm total length) of yellow perch showed decreased growth rates when a predator (walleye, *Sander vitreus*) was first introduced; however, growth rates increased after the predator became well established (Olson et al. 2001).

Behavioral changes related to predation pressure can influence the swimming ability in prey. Swimming performance is closely related to morphological structure and body shape but is also dependent on muscle tissue capacities for aerobic and anaerobic activities (Webb 1976; Domenici 2001; Blake 2004). Anaerobic enzyme activities, or burst swimming activity, have been commonly estimated by the activity rates of the glycolytic enzyme lactate dehydrogenase (LDH) (Sherwood et al. 2002). The enzyme activity rate of a mitochondrial enzyme, citrate synthase (CS), has also been used to assess aerobic or sustained swimming activity, but this enzyme has not been widely used in wild populations (Rajotte and Couture 2002). In addition to swimming activity, enzymatic activity rates have also been used to estimate growth potential in fishes by nucleoside diphosphate kinase (NDPK), an indicator of protein synthesis (Couture et al. 1998; Rajotte and Couture 2002). One would therefore expect low NDPK levels to occur in slow-growing stunted yellow perch populations (Olson et al. 2001).

Phenotypic divergences in terms of shape changes have been well documented in perch and consist of two rather distinct morphologies: an open-water form (including limnetic or pelagic forms) and a shallow-water form (including benthic and littoral forms) (Schluter 1996; Proulx and Magnan 2002; Svanbäck and Eklöv 2002). The shallow-water form typically has a deep body, a downwards-bending snout, and long pectoral fins. This form appears to be adapted for feeding on macroinvertebrates and other littoral zone prey. Open-water forms have an upward-pointing snout and a more slender body shape. This body conformation may decrease the amount of drag produced when swimming and is thus more energetically suitable for feeding on planktonic prey items (Svanbäck and Eklöv 2003). Such trophic polymorphisms are particularly common in fish and bird species that use their mouths to capture and hold their prey (Wimberger 1994).

This study examines the effects of predator invasion on yellow perch populations in lakes from the Sudbury area recovering from acidification. Acid and metal deposition from the mining industry that began in the 1880s caused extensive biological damage to area lakes, including the loss of many acid-sensitive predator populations (Matuszek et al. 1992). The removal of the top predator left many lakes with very simple fish communities, often consisting largely of abundant populations of yellow perch (Gunn et al. 1990). Large reductions (~90%) in sulphur and metal emissions from local smelters in recent decades have allowed chemical recovery in Sudbury lakes to occur (Gunn and Keller 1990), permitting the reestablishment of indigenous predators (such as walleye and northern pike (*Esox lucius*)). At the same time, the expansion of smallmouth bass (*Micropterus dolomieu*) into the Sudbury area has occurred. Smallmouth bass is a warm-water invasive species at the northern edge of its distribution in the Sudbury lakes and now appears to be responding to climate-induced changes by increased overwinter survival of young (Dunlop and Shuter 2006).

Our objective was to make use of this unique opportunity to study the early stages of predator colonization in these industrially stressed lakes to assess the effects of invading predators on yellow perch populations. In lakes with predators present, we hypothesized that perch would exhibit (*i*) a decrease in the abundance, (*ii*) increased growth and investment in reproduction, (*iii*) early ontogenetic diet shifts, (*iv*) decreased swimming activity (by enzyme analysis), and (*v*) a change in body shape to reduce vulnerability by gape-limited predators.

Materials and methods

Study lakes

Yellow perch populations from 18 lakes within the City of Greater Sudbury, Ontario, were selected for this study (Table 1; Fig. 1). The lakes were relatively small (surface area 20–1079 ha; median 77 ha) and were all underlain by Precambrian Shield bedrock. However, for Shield lakes they did vary quite widely in limnological characteristics such as maximum depth (6.0–50.3 m), water clarity (Secchi 2.4–14.0 m), base cation (Ca 1.4–16.6 mg·L^{-1}) and nutrient (total P 2.7–24.3 µg·L^{-1}; DOC 1.9–8.8 mg·L^{-1}) concentrations (Table 1), and fish community composition (Table 2). With the exception of Lovering Lake (36 km from smelter), all of the lakes were within 30 km of Sudbury, in the area of high metal particulate deposition, and as a result, most lakes had metal concentrations of Cu and Ni well above the provincial water quality objectives (PWQO) (5 µg·L^{-1} Cu; 25 µg·L^{-1} Ni; Table 1). Many of the lakes were very well studied in that the history of chemical recovery (i.e., pH ↑, metals ↓) and the changes in the fish communities since 1990 were known (Keller et al. 1992; Gunn and Keller 1995; J.M. Gunn, unpublished data). Introduction of predators such as smallmouth bass and walleye occurred as a result of hatchery stocking by government agencies or by unrecorded introductions by citizen groups or by natural movements between lakes during high-water periods. Based on the results of netting surveys (discussed later), the lakes were classified into three different predator regimes: 1, lakes with no predators (Camp, Clearwater, Crowley, Linton, Lohi, and Wavy lakes); 2, lakes with recently (≤3 years) introduced predators (Daisy, Hannah, Middle, and St. Charles lakes); and 3, lakes with well-established (≥15 years) predator populations (Lovering, Massey, McFarlane, Nelson, Nepahwin, Ramsey, Vermilion, and Whitson lakes).

There were no significant differences in maximum or mean depth or Secchi depth transparency among the lake groupings (Table 1). However, the lakes with established predators had significantly larger surface area, a fact that may also explain the higher fish species richness in these lakes (Table 2). In terms of chemical conditions, the predator groupings correspond well with the stage of chemical recovery or the overall productivity of the lakes. For example, lakes that lacked predators had lower pH, P, Ca, and DOC than the lakes with established predator populations (Table 1). The

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Table 1. Selected physical and chemical characteristics for the 18 study lakes from the Sudbury area grouped by predator regime.

Predator regime	Lake	Latitude (N)	Longitude (W)	Surface area (ha)	Maximum depth (m)	Mean depth (m)	Secchi depth (m)	pH 1990	pH 2004	P (μg·L^{-1})	Ni (μg·L^{-1})	Cu (μg·L^{-1})	Ca (mg·L^{-1})	DOC (mg·L^{-1})
No predators	Camp[†]	46°22′58″	81°00′10″	20	15.0	8.7	4.0	6.30[*]	6.69	4.0	50.6	7.4	2.4	2.6
	Clearwater	46°22′13″	81°03′01″	77	20.4	8.2	5.7	4.88	6.27	4.2	60.4	10.1	4.0	2.6
	Crowley	46°23′00″	80°59′00″	42	16.0	6.4	5.2	5.88	6.12	7.8	52.6	10.2	2.0	2.2
	Linton	46°22′00″	80°59′00″	27	16.0	6.4	5.8	5.41	5.97	6.6	55.7	10.4	1.8	2.5
	Lohi	46°23′00″	81°02′00″	41	18.5	6.2	6.3	4.92	6.28	7.1	56.3	11.0	4.0	2.8
	Wavy	46°18′00″	81°06′00″	306	34.0	15.0	3.5	4.74	5.11	3.7	34.7	5.6	1.4	2.2
	Group mean	—	—	85.5a	20.0a	8.5a	5.1a		6.07a	5.6a	51.7ab	9.1a	2.6a	2.5a
Recent predators	Daisy	46°27′00″	80°54′00″	36	14.0	5.2	14.0	4.82	6.52	6.7	62.2	8.0	2.4	1.9
	Hannah	46°27′00″	81°02′00″	27	8.5	4.0	3.3	7.06	7.32	7.6	94.7	22.1	10.8	3.8
	Middle	46°26′00″	81°02′00″	28	15.0	6.2	5.4	6.57	7.09	6.7	95.5	24.0	8.8	3.4
	St. Charles	46°26′00″	81°01′00″	41	6.0	3.0	3.5	7.11	7.12	11.6	100.0	25.7	8.9	4.1
	Group mean	—	—	33.0a	10.9a	4.6a	6.6a		7.01b	8.2a	88.1b	19.9a	7.7ab	3.3ab
Established predators	Lovering[†]	46°13′55″	80°45′12″	90	20.0	7.3	2.5	—	7.65	13.0	15.0	4.0	3.8	8.8
	Massey[†]	46°40′19″	80°51′10″	77	4.0	11.1	2.7	—	6.47	18.0	16.3	7.3	2.8	5.4
	McFarlane	46°25′01″	80°57′23″	141	20.1	5.8	2.4	7.10	7.21	21.0	53.7	8.7	15.8	4.3
	Nelson	46°43′36″	81°05′40″	316	50.3	9.3	8.2	6.43	6.64	2.7	4.6	2.1	2.2	3.0
	Nepahwin	46°27′00″	80°58′00″	128	22.0	8.6	8.2	7.71	7.58	17.0	49.2	10.5	16.6	4.3
	Ramsey	46°28′57″	80°57′01″	874	21.3	2.2	3.8	7.48	7.32	14.6	62.5	12.8	14.1	3.1
	Vermilion[†]	46°31′22″	81°23′28″	1079	13.1	4.7	3.7	—	7.31	24.3	12.0	3.2	10.1	5.2
	Whitson	46°35′00″	80°58′00″	437	16.0	7.3	3.6	5.97	6.92	14.0	92.2	19.7	8.4	2.8
	Group mean	—	—	392.8b	20.8a	7.0a	4.4a	—	7.14b	15.6b	38.2a	8.5a	9.2b	4.6b

Note: The chemical data was provided by OMOE from midlake epilimnetic samples collected in 2004. Available pH values from 1990 also indicated. Group means within columns followed a common lowercase letter are not significantly different (analysis of variance (ANOVA); least significant difference (LSD)). DOC, dissolved organic carbon.

[*]Mean of four monthly samples from summer 1992 (Wright 1995).

[†]Samples taken during summer of 2006 and analyzed at Test-Mark Laboratories in Sudbury.

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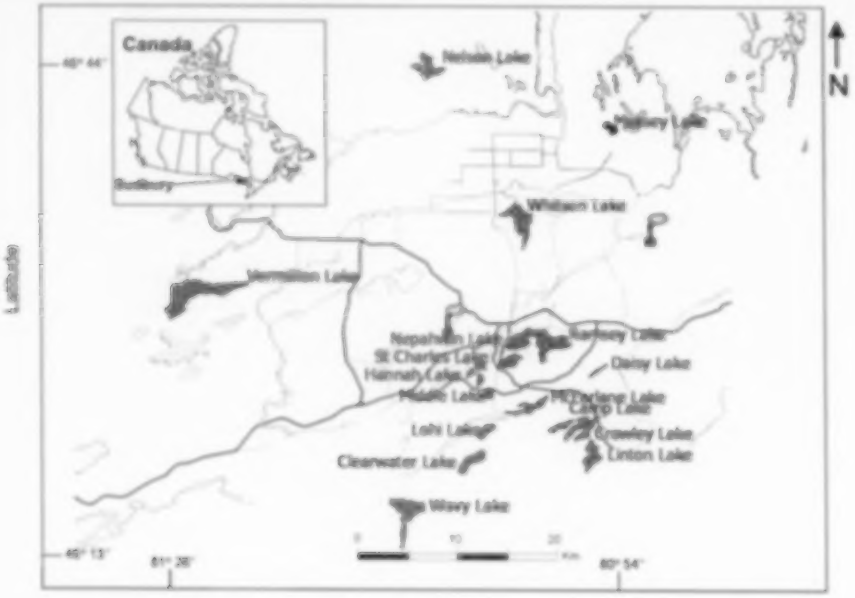
Fig. 1. Map showing the location of each study lake (except Lovering Lake, an established predator lake, which is south of the display area). The smokestack icons indicate the locations of the two active metal smelters.



recently invaded lakes were intermediate in the variables related to acidification and overall productivity, but they did have elevated metal (Cu, Ni) concentrations compared with the other two groups. Metals may therefore have some confounding effects on the biota in the lakes where the predators first arrive (will be discussed later).

Sampling

Standard netting method

Fish community assessments of all study lakes were conducted in the summers of 2004 and 2005 using the Nordic multimesh gillnetting method (Appelberg 2000) (Table 2). This quantitative, depth-stratified method uses a geometric series of multimesh gill nets (5–55 mm) with a sampling intensity scaled for lake area and maximum depth. The nets were oriented randomly with respect to the shoreline contour between 1800 and 2000 and then were picked up the next morning between 0600 and 0800. The lifted nets were stored in plastic bins and transported to the Ontario Ministry of Natural Resources (OMNR) necropsy laboratory and processed within 8 h of lifting. Fish were enumerated, and each individual was measured for fork and total length (±1 mm) and body mass (±0.01 g). Data were used to estimate relative abundance, spatial distributions, and biomass ($g \cdot net^{-1}$) for each fish species. In addition, samples of perch from the catch of each lake (target of 20 fish for every 10 mm size class) were frozen for later stomach content analysis.

Additional fall sampling

Yellow perch were collected at the end of the growing season in October 2004 from each of the study lakes to obtain samples for muscle tissue, body shape, and life history analysis, with the exception of Camp, Crowley, Linton, and Nepahwin lakes, which were only sampled for body shape and life history analysis in 2005 (Table 3). Benthic gillnets (9.5 and 12.5 mm knot-to-knot) were set in depths between 2 and 5 m for approximately 24 h. These mesh sizes were chosen to target a similar perch size range (90 to 130 mm in total length) in each lake. Captured fish were measured for total length (±1 mm) and body mass (±0.01 g).

A subsample of perch was selected for analysis of body shape by choosing the first 30 individuals between 90 and 130 mm in total length with no external physical damage (e.g., broken opercle bone). Afterwards, sex and maturity were determined by visual inspection of gonads, first in fish sampled for body shape analysis and then in additional perch (to a maximum of 20 males and 20 females) of similar size (90 to 130 mm total length) used for metabolic enzyme analysis. Muscle plugs were removed from the males (n = 20) and females (n = 20) and stored in liquid nitrogen <4 h after the perch were removed from the lake. Greater details describing the methods used for body shape and metabolic enzyme analysis will be presented later. Finally, a subsample (n = 100) from each lake was processed for life history analysis ensuring that an equal number of each size class (using 10 mm size classes) was collected across the available range. All mature gonads were weighed (±0.01 g wet mass), and mature ovaries were preserved in 95% ethanol for later fecundity estimates. Saggital otoliths were removed and age was interpreted by a single observer (S. Mann, Ontario Ministry of Natural Resources, Dryden, Ontario) by cracking and burning and counting winter bands (Christensen 1964).

Additional spring sampling

Mature female perch were collected just before spawn in April 2005 from a subset of the study lakes (Table 3) to as-

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Table 2. Fish community characteristics for the 18 study lakes based on summer Nordic netting assessment.

			Predator species[†]										
Predator regime	Lake	Species richness[*]	LT	NP	SMB	SP	W	Predator biomass (g·net^{-1})[‡]	Perch biomass (g·net^{-1})[‡]	Cyprinid biomass (g·net^{-1})[‡]	Total biomass (g·net^{-1})[‡,§]	Total no. of nets	Nordic netting assessment (month/year)
No predators	Camp	2						0.0	759.1	0.0	760.1	16	07/2005
	Clearwater	3						0.0	1248.7	4.4	1257.9	24	08/2004
	Crowley	1						0.0	2121.2	0.0	2121.2	16	07/2005
	Linton	2						0.0	2111.4	0.0	2112.2	14	07/2005
	Lohi	3						0.0	1746.0	10.5	1757.7	16	09/2004
	Wavy	1						0.0	998.2	0.0	998.2	47	08/2004
Recent predators	Daisy	7		2			1	205.0	978.9	1.7	1343.9	16	07/2004
	Hannah	6		2	1			247.3	768.8	0.0	1940.9	16	09/2004
	Middle	7		1	1		1	286.4	382.5	0.0	675.6	16	09/2004
	St. Charles	5		7			7	840.4	648.6	0.0	1723.0	8	08/2004
Established predators	Lovering	10		1	15		30	545.8	36.5	0.0	1729.6	32	07/2004
	Massey	5		31			31	1356.4	1210.1	13.6	3543.0	16	07/2004
	McFarlane	13		12	75		25	956.8	156.9	36.0	3831.2	32	08/2004
	Nelson	11	67	[¶]	62			917.8	302.1	1.2	1547.3	48	08/2004
	Nepahwin	11		2	73	16		944.1	170.3	0.0	2702.0	32	07/2004
	Ramsey	8		7	49		178	886.0	80.7	0.0	1849.0	47	07/2004
	Vermilion	14		22	12		251	1724.5	385.2	19.8	4721.0	32	07/2004
	Whitson	7		17	1		135	1268.2	516.0	0.0	2468.2	40	07/2004

[*]Total number of fish species collected during the Nordic Lake survey.
[†]Total number of individuals collected during the Nordic survey. Predator species are represented by the following acronyms: lake trout (LT), northern pike (NP), smallmouth bass (SMB), splake (SP), and walleye (W).
[‡]Mean biomass calculated from the individual weights of fish captured per net during the Nordic survey.
[§]Total biomass included other fish species not included in predator, perch, and cyprinid biomass estimates.
[¶]Present in the lake but not captured during the 2004 assessment.

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Table 3. Sampling times for the 18 study lakes.

			Fall sampling*			Spring sampling
Predator regime	Lake	Yellow perch diet analysis (month/year)	Life history	Metabolic enzyme	Body shape	Lipid
No predators	Camp	07/2005	×		×	
	Clearwater	08/2004	×	×		×
	Crowley	07/2005	×		×	
	Linton	07/2005	×		×	
	Lohi	09/2004	×	×	×	×
	Wavy	08/2004	×	×		×
Introduced predators	Daisy	07/2004	×	×	×	
	Hannah	09/2004	×	×	×	×
	Middle	09/2004	×	×	×	×
	St. Charles	08/2004	×	×	×	×
Established predators	Lovering	07/2004	×	×		
	Massey	07/2004	×	×		
	McFarlane	08/2004	×	×		×
	Nelson	08/2004	×	×	×	
	Nepahwin	07/2004	×		×	×
	Ramsey	07/2004	×	×	×	
	Vermilion	07/2004	×	×		
	Whitson	07/2004	×	×	×	

*Fall samples for detailed body shape, metabolic enzyme activity rates, and life history analyses were completed in October of the same year as the Nordic assessment, except for Nepahwin Lake, which was sampled in October 2005.

sess lipid content of eggs. The same netting procedure and sampling protocol as used during the fall period were used. Mature females were measured for total length (±1 mm) and body mass (±0.01 g). Sex and maturity were assigned by visual inspection of gonads. Ovaries were removed, weighed (±0.01 g), and frozen (–20 °C) for later lipid extraction.

Diet analysis

Perch collected during the summer Nordic lake surveys (target of 20 fish for every 10 mm size class) were frozen for later diet analysis (Table 3). Once thawed, each fish was measured for total length (±1 mm) and body mass (±0.01 g), and sex was determined. Stomachs were opened and food items were transferred to a clean Petri dish and diluted with water to determine the presence or absence of zooplankton, benthos, and fish in the diet.

Reproductive tissue analysis

Mature ovaries from the spring samples were used for lipid analysis (Table 3). Ovaries were freeze-dried for 7 days, and three subsamples of 30 freeze-dried eggs were weighed (±0.1 mg) to determine mean egg size (mg dry mass·egg^{-1}). The ovary was then ground to a powder with a mortar and pestle. Lipid extraction methods were modified from Folch et al. (1957) and Herbes and Allen (1983). Weighed subsamples (~ 0.20 g) of ground tissue were extracted twice with chloroform–methanol (2:1 by volume) solvent, and the supernatant was washed with 0.88% KCl (aqueous). The solvent layer (containing lipid) was transferred to preweighed aluminum pans. Pans were dried in a fume hood until all solvent was evaporated and then reweighed to determine mass of lipid extracted. Lipid content per egg (mg·egg^{-1}) was then estimated as ovary lipid concentration (g extracted lipid·(g ovary subsample)$^{-1}$) multiplied by mean egg size (mg dry mass).

Enzyme analysis

Fish from lakes sampled in the fall of 2004 (Table 3) were used for muscle enzyme analysis. Samples were restricted to a single season and year to avoid potential interannual variation (Leonard and McCormick 1999). To control for variation in enzyme activity rates associated with fish size and sex (Pelletier et al. 1995), all sampled fish were between 90 and 130 mm in total length and equal sample sizes of males and females (20 each) were used. White muscle samples (~0.50 g) were removed from the left caudal region of each specimen (Leonard 1999) and immediately frozen in liquid nitrogen and stored at –80 °C prior to enzyme assays. Enzyme (LDH, CS, and NDPK) activity rates were measured in duplicate (triplicate if greater than 5% difference between replicates) for 5 min using a temperature-controlled spectrophotometer (Varian Cary 100; Varian Inc., Palo Alto, California) at 18 °C. Detailed methods are outlined in Rajotte and Couture (2002).

Shape analysis

Yellow perch sampled in the fall from four lakes within each predator regime were selected for shape analysis (Table 3). In each lake, the first 30 perch of the targeted size range (90 to 130 mm total length) removed from the nets were put aside for photography before further dissection. Each perch was placed on a mat with 1 cm^2 grids with the anterior tip of the maxilla and fork of the tail aligned as a straight line on the grid (Fig. 2). The caudal fin was gently

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pressed against the grid to ensure visibility of the fork of the tail. The surface of the perch was lightly blotted with a towel to minimize glare before the photograph was taken. Digital images (Nikon COOLPIX880, 3.34 megapixels) were taken of the left lateral side of each fish. Total length (±1 mm) and body mass (±0.01 g) were measured, and sex was recorded for each individual after the image was taken. All fish preparations, photography, and specimen selection were conducted by the same person (K.A. Lippert) following a standard protocol.

Individual digital photos were appended using tpsUtil (Rohlf 2004) and imported into tpsDig (Rohlf 2005*a*). Sixteen homologous landmarks (Fig. 2) were chosen as common fixed morphological features of perch (Svanbäck and Eklöv 2003) and other fish species (Corti and Crosetti 1996; Hard et al. 2000; Loy et al. 2000). Landmarks were digitized for all specimens by the principal author (K.A. Lippert) and imported into tpsRelw (Rohlf 2005*b*) to conduct geometric morphometric analysis. Landmarks were used to create a configuration matrix where a series of translations were computed to remove differences in location, size, and rotation for each individual. Eigenvalue analysis on the configuration matrix was performed to calculate 26 partial warps and two uniform scores. To visualize the deformations in shape between groups of individuals, thin-plate splines were generated using tpsRegr (Rohlf 2003).

Statistical analysis

Physical and chemical lake properties were compared among the predator regimes using analysis of variance (ANOVA) followed by a comparison of means using Fisher's least significant difference (LSD).

Yellow perch biomass (g·net^{-1}) was compared among predator treatment groups using ANOVA. The presence or absence of prey categories (zooplankton, benthos, fish) in perch stomachs was used to generate logistic regressions for each predator regime. The presence or absence of any particular prey type was not dependent on another prey type being present or absent. Logistic regressions of the form

$$(1)\quad y = e^{a+bx}/(1 + e^{a+bx})$$

were fitted for each of the three prey categories, where y is the probability of finding the prey type in the perch stomach (0 or 1), x is perch length, and a and b are fitted parameters (Tolonen et al. 1999). Models were fitted to data from individual lakes and then to pooled data from all lakes within predator regimes and used to estimate ontogenetic diet shifts (i.e., size-at-50% probability) for each food category by predator group.

Log-transformed total length (mm) was significantly correlated with log-transformed body mass (g), gonad weight (g), fecundity (number of eggs·individual^{-1}), egg mass (mg·egg^{-1}), and egg lipid content (mg·egg^{-1}) for each lake. Estimated values for each of the listed life history variables were adjusted to a common covariate total length across all lakes. Estimated life history parameters for each lake were used to test for differences among predator groupings using ANOVA. Using the fall sample, average total lengths (mm) of young-of-year (YOY) and 1- and 2-year-old individuals (calculated separately for males and females in each lake) were compared by ANOVA among the predator regimes.

Fig. 2. Landmark locations used for yellow perch morphological analysis in 12 of the study lakes. Positions of landmarks are as follows: (1) tip of the dentary; (2) operculum–isthmus overlap; (3) insertion of pelvic fin; (4) tip of pelvic fin; (5) insertion of anal fin; (6) tip of anal fin; (7) posterior–ventral edge of caudal peduncle; (8) posterior end of hypural bones; (9) posterior–dorsal edge of caudal peduncle; (10) end of soft-ray dorsal fin; (11) insertion of soft-ray dorsal fin; (12) insertion of spiny-ray dorsal fin; (13) tip of maxilla; (14) centre of eye; (15) tip of operculum spine; and (16) fork of caudal fin.

Power analysis was preformed on life history parameters where no differences among lake groups were detected.

Mean metabolic enzyme capacities (e.g., LDH, CS, and NDPK) were calculated for each lake and were used in an ANOVA to test for differences in swimming activity among the predator regimes. All enzyme activity rates were log-transformed to normalize residuals.

The morphological index (MI) is the function produced when partial warp and uniform scores were analyzed using discriminant function analysis (DFA). This methodology for calculating MI has been used to discriminate individuals belonging to different predator regimes (Proulx and Magnan 2002).

Principal component analysis was used to calculate significant explanatory factor scores for each lake using all physical and chemical variables (lake environmental characteristics) independently. The environmental factor scores, biomass estimates (yellow perch, predators, and fish community), and biological variables were compared using correlation analysis.

All statistical analyses were completed using STATISTICA software (StatSoft Inc., Tulsa, Oklahoma) and considered to be significant if $p < 0.05$.

Results

Fish biomass

Yellow perch biomass was significantly lower (69%) in lakes with predators than in lakes with no predators (ANOVA, $F_{[2,15]} = 11.8$, $p < 0.001$). However, perch biomass did not differ significantly between groups of lakes with recently invaded versus established predators (Fig. 3). In the absence of predators, yellow perch biomass averaged 1497.4 g·net^{-1} (±580.4 standard deviation (SD), $n = 6$), whereas in lakes with recently introduced and established predators, the perch biomass was 694.7 g·net^{-1} (±978.9 SD, $n = 4$) and 357.2 g·net^{-1} (±380.0 SD, $n = 8$), respectively (Table 2).

Lakes with established predator populations had significantly higher total biomass than the other two predator groupings (ANOVA, $F_{[2,15]} = 5.07$, $p = 0.021$). However, to-

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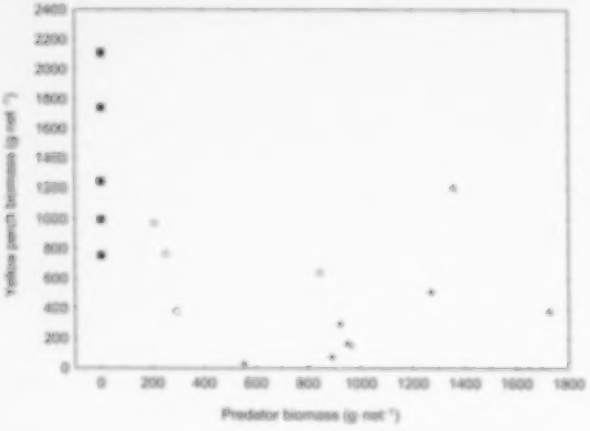
Fig. 3. Relationship between yellow perch biomass (g fish·net^{-1}) and predator biomass (g fish·net^{-1}) for 18 lakes sampled in Sudbury, Ontario: ■, lakes with no predators; ◇, lakes with recently invaded predators; +, lakes with established predators.



tal fish biomass correlated positively with predator biomass (linear regression, $R = 0.79$, $p < 0.001$; total community biomass = 1267.89 + 1.55(predator biomass)). When adjusted for predator biomass, total fish biomass did not differ across the predator regimes (analysis of covariance, ANCOVA, $F_{[2,15]} = 1.42$, $p = 0.274$).

The total biomass of forage fish for each lake was estimated from surveys by summing the biomass of yellow perch and cyprinid species in the Nordic nets. Irrespective of lake grouping, yellow perch was found to dominate (>80%) the available forage fish biomass in all study lakes. The proportion of yellow perch biomass (as a function of the total forage fish biomass) did not differ significantly across the predator regimes (ANOVA, $F_{[2,15]} = 1.03$, $p = 0.381$).

Ontogenetic diet shift

The perch from lakes with predators displayed a diet shift from zooplankton to benthos at a much smaller size (83 mm) compared with perch from lakes with no predators (140 mm) (ANOVA, $F_{[1,7]} = 7.48$, $p = 0.029$) (Table 4). We defined the point of ontogenetic diet shift as the perch length corresponding to the inflection point in the fitted logistic regressions (i.e., prey prevalence = 50%) (Table 4; Fig. 4). Ontogenetic diet shifts onto fish occurred at 200 and 217 mm in total length in lakes with no predators and recently invaded predators, respectively (Fig. 4). However in lakes with established predator populations, the predicted size (i.e., 268 mm) at which perch shifted their diet to fish was larger than the maximum size of perch sampled in this group of lakes (Fig. 4c). Therefore, perch from lakes with established predators did not completely make the last ontogenetic shift to fish.

Reproductive investment

Male perch had higher reproductive investment in the absence of interspecific predators (Table 5). The estimated testes mass (0.95 g) for a 110 mm perch in lakes with no predators was significantly higher (ANOVA, $F_{[2,15]} = 5.43$, $p = 0.017$) than in lakes with recently introduced predators (~0.70 g) and lakes with established predators (~0.77 g).

Table 4. Predicted total lengths (mm) of yellow perch at 50% probability of finding a prey category (zooplankton, benthos, fish) in stomach contents.

		Yellow perch length (mm)		
Predator regime	Lake	Zooplankton	Benthos	Fish
No predators	Camp	132	130	183
	Crowley	163	167	209
	Linton	134	—	231
	Lohi	90	—	179
	Wavy	114	—	176
	Clearwater	139	123	198
Introduced predators	Daisy	132	95	202
	Hannah	101	78	208
	Middle	76	22	186
	St. Charles	78	—	160
Established predators	Nelson	95	—	136
	Nepahwin	138	105	—
	Ramsey	118	101	—
	Whitson	107	98	152

Note: Only those lakes with significant logistic regressions are included. Predicted lengths were derived from fitted logistic regressions for each study lake.

Reproductive investment of females also varied with respect to predator status; however, the direction was opposite to that of the males. Females had higher reproductive investment in lakes with established predators compared with the other two lake groupings. Reproductive investment, defined as the weight of ethanol-preserved fall ovaries adjusted to a standard length of fish, was highest in lakes with established predators (ANOVA, $F_{[2,15]} = 12.06$, $p < 0.001$; Table 5). In lakes with established predators, the mean estimated preserved fall ovary mass for a 125 mm long perch was 0.64 g. Yellow perch from lakes with no predators and recently introduced predators had adjusted mean ovary masses of 0.43 and 0.50 g, respectively (not statistically different from each other).

Female perch were also found to make a trade-off between ovary size and the lipid content per egg (Table 5). The spring lipid content of eggs from perch in lakes with recently invaded predators was significantly lower than in perch from lakes with no predators (ANOVA, $F_{[2,5]} = 7.0$, $p = 0.036$). There was, however, no statistical difference in egg lipid content among lakes with established predators and either of the other two predator regimes, likely because of low statistical power ($1 - \beta = 0.069$, where β is the probability of a type II error) with such a small number of lakes (266 lakes are required for $\beta = 0.80$). Perch egg lipid content in lakes with no predators, recently invaded predators, and established predators was 0.040, 0.027, and 0.034 mg·egg^{-1}, respectively.

Perch length-at-age

Both male and female YOY perch in lakes with recently introduced predators were significantly smaller (13%) at the end of their first growing season than YOY perch in lakes with no predators (ANOVA, $F_{[2,29]} = 5.02$, $p = 0.015$; Ta-

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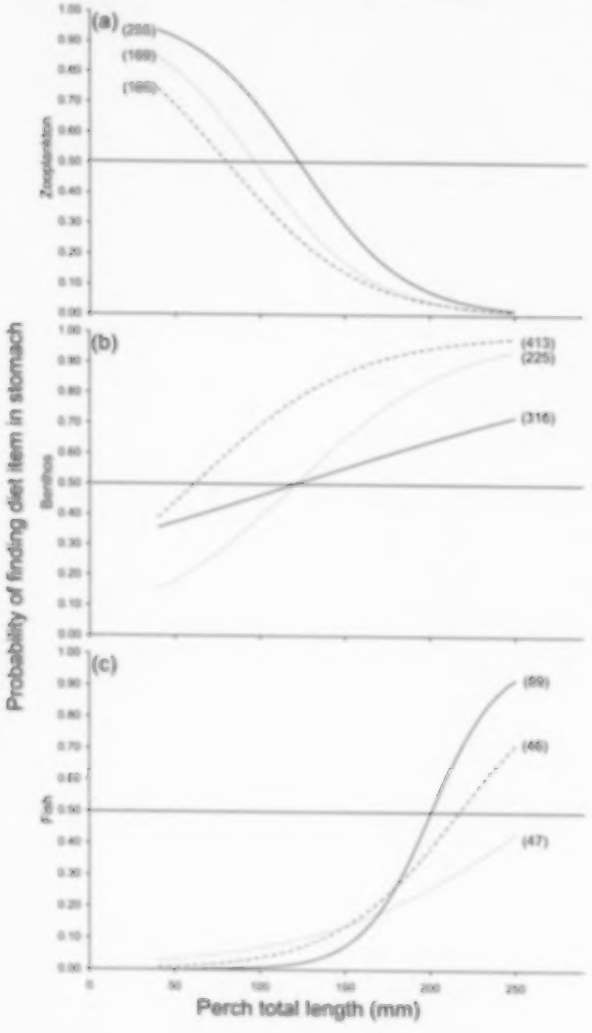
Fig. 4. Total length of perch at the point where 50% of the sampled fish contained (*a*) zooplankton, (*b*) benthos, and (*c*) fish in their stomachs in lakes with no predators (solid line), lakes with recently introduced predators (broken line), and lakes with established predators (dotted line). All the fish were collected during the summer Nordic lake survey. The number of stomachs in the logistic regressions containing the diet item is indicated in parenthesis.



ble 5). Similar to the egg lipid effects, this response to predators also seemed transient; growth of YOY perch from lakes with well-established predator populations was not statistically different from YOY growth in lakes with no predators or recently introduced predators.

Not all lakes within a single predator grouping were sampled within the same year, so interannual variation was tested within each regime. There was no significant difference between the total length of YOY perch (males, p = 0.477; females, p = 0.414) in lakes with no predators sampled in the fall of 2004 and 2005. Similarly, no interannual differences were found for age-1 (male, p = 0.667; female,

Table 5. Summary of the life history and metabolic enzyme activity rates from yellow perch populations across the predation regime.

	Predator regime						
	Female			Male			
Variable	No	Recent	Established	No	Recent	Established	*p*
Round weight (g)	11.04a (±0.61)	10.86a (±0.84)	12.18b (±0.82)	11.61a (±0.52)	11.03a (±0.86)	12.43b (±0.74)	<0.001
Gonad weight (g)	0.44a (±0.06)	0.51a (±0.08)	0.66b (±0.09)	0.92b (±0.09)	0.69a (±0.12)	0.76a (±0.11)	0.017*
							<0.001†
Egg lipid content (mg·egg^{-1})	0.041b (±0.007)	0.025a (±0.009)	0.036ab (±0.033)	—	—	—	0.036‡
Length-at-YOY (mm)	68b (±8)	61a (±5)	68ab (±8)	74b (±7)	61a (±4)	66ab (±8)	0.015
Length-at-age 1 (mm)	105 (±5)	108 (±11)	108 (±13)	98 (±3)	99 (±7)	105 (±13)	0.629
Length-at-age 2 (mm)	147b (±26)	139b (±21)	147b (±17)	129a (±21)	118a (±31)	125a (±15)	0.654
NDPK (IU)	15.05a (±4.73)	11.91a (±5.77)	26.27b (±7.23)	13.87a (±5.37)	13.57a (±4.76)	21.09b (±9.30)	0.007
LDH (IU)	159.49 (±95.55)	128.98 (±21.36)	127.40 (±36.79)	169.60 (±99.82)	136.42 (±37.32)	128.49 (±34.04)	0.270
CS (IU)	0.84 (±0.325)	0.69 (±0.237)	0.53 (±0.212)	1.01 (±0.639)	0.70 (±0.162)	0.60 (±0.268)	0.128

Note: Data represent means (±95% confidence intervals) from logarithmically transformed estimates using 110 mm (males) and 125 mm (females) as covariates (values determined by taking the mean of the intersecting ranges among all lakes). p values are shown for two-way analysis of variance (ANOVA) analysis with the predator regime and sex as the treatment groupings where there was no significant interaction. Statistically similar values are denoted by the same lowercase letter. YOY, young of the year; NDPK, nucleoside diphosphate kinase; LDH, lactate dehydrogenase; CS, citrate synthase; IU, international units.

*Results from one-way ANOVA analysis for each sex (there was a significant interaction between predator regime and sex in the two-way ANOVA analysis).

†Results from one-way ANOVA.

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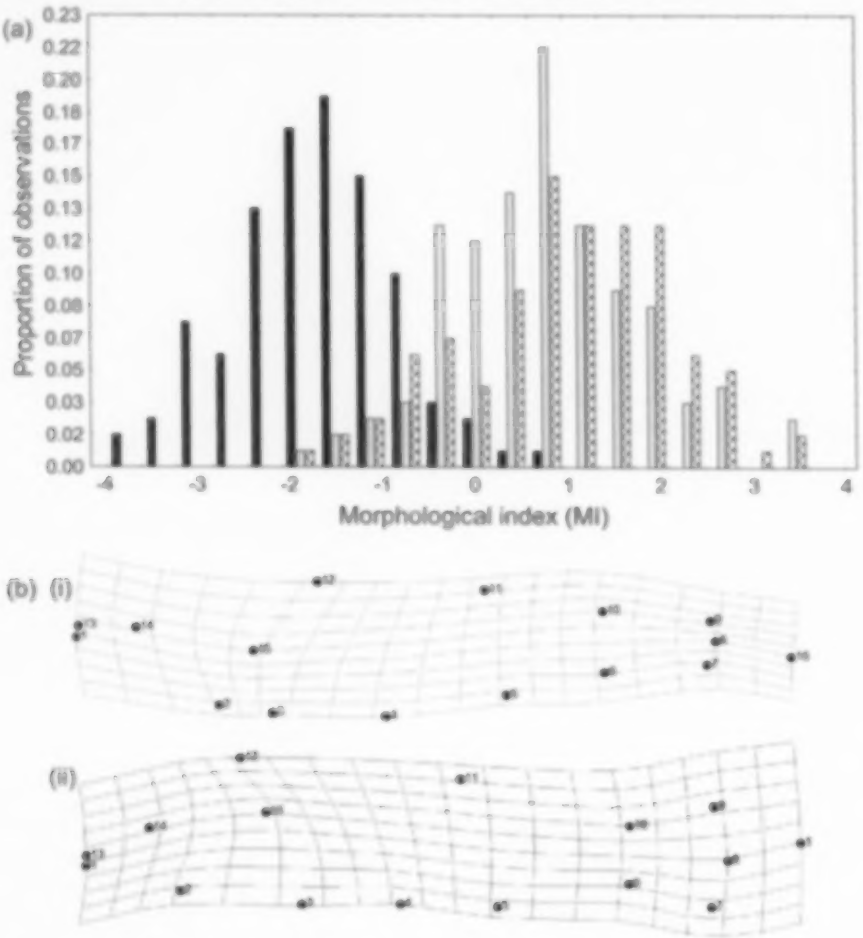
Fig. 5. Morphometric analysis. (*a*) Distribution of yellow perch morphological index (MI) (determined by discriminant function analysis of partial warp scores) from lakes with no predators (solid bars), recently invaded predators (shaded bars), and established predators (cross-hatched bars). (*b*) Deformation grids depicting shape differences for a typical perch in a lake with (*i*) no predators and (*ii*) established predators are shown. Numbers identify the landmarks used in the analysis as described in Fig. 2. The distribution of the morphological index (MI) shows that perch in lakes without predators were at the left (negative) side of the distribution, established predators were at the right (positive) side, and introduced predator exposure was intermediate between the two.



$p = 0.431$) or age-2 (male, $p = 0.364$; female, $p = 0.147$) individuals for this grouping.

In contrast to the effects seen in growth during the first year of life, size-at-age for older age classes of perch did not vary greatly across the predator regimes. At age 1+, perch size did not differ between males and females (ANOVA, $F_{[1,30]} = 2.29$, $p = 0.141$) or across the predation gradient (ANOVA, $F_{[2,60]} = 0.47$, $p = 0.629$; Table 5). By age 2, typical sexual dimorphisms in perch were established; females were 13% larger on average than males across the predation gradient (ANOVA, $F_{[2,27]} = 7.49$, $p = 0.011$; Table 5).

Metabolic enzyme activity rates and body condition

Analysis of muscle NDPK indicated that perch in lakes with established predator populations had higher growth potential than perch in lakes with either no predators or only recently introduced predators (ANOVA, $F_{[2,22]} = 6.28$, $p = 0.007$; Table 5). Mean NDPK rates were 23.44, 14.45, and 12.72 IU·(g wet mass)$^{-1}$ in lakes with established predators, lakes with no predators, and lakes with recently introduced predators, respectively.

Body condition, quantified as total body mass adjusted for length, was not statistically different between lakes without predators and recently invaded predators but was higher in males and females in lakes with established predator populations (ANOVA, $F_{[2,30]} = 10.48$, $p < 0.001$; Table 5). This trend was similar to the effect observed in growth potential as estimated by NDPK activity. The higher growth potential in lakes with established predators therefore seems to be directed towards increased weight at length (i.e., condition) rather than increased total length of fish.

Metabolic enzyme estimates for swimming activity

Yellow perch LDH rates (log-transformed) did not differ significantly among predator groupings (ANOVA, $F_{[2,22]} = 1.40$, $p = 0.270$; Table 5). On average, the LDH rates from

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perch between 100 and 120 mm total length were 134.9 IU·(g tissue wet mass)$^{-1}$. Similarly, CS rates (log-transformed) of yellow perch did not differ significantly across the predator groupings (ANOVA, $F_{[2,22]} = 2.26$, $p = 0.128$; Table 5). The average CS rate was 0.66 IU·(g tissue wet mass)$^{-1}$. The lack of difference in LDH and CS rates across the predator groupings suggests that the addition of interspecific predators did not change anaerobic or aerobic swimming potentials in yellow perch.

Body shape analysis

The body shape of the perch could be readily used to classify the predator regime from which they came. From the morphometric analysis, 86.1% of individuals was correctly classified into the appropriate predator regimes (Fig. 5*a*).

Perch in lakes without predators had shallow bodies, a slightly upturned snout region, and a thin, elongated caudal peduncle region (landmarks 6, 7, 8, 9, and 10) (Fig. 5*b* (*i*)). Perch in lakes with predators had thicker bodies with heads pointed slightly upwards and a thick, short caudal peduncle (Fig. 5*b* (*ii*)). The insertion of the spiny dorsal fin (landmark 12) was more anterior and the pelvic fin insertion (landmark 3) was more posterior compared with perch in lakes with no predators.

The population mean MI increased rapidly with predator biomass (Fig. 6*a*), up to approximately 200–400 g·net^{-1}. Beyond this level of predator biomass, body morphology did not change significantly. Mean population MI also decreased significantly with increasing perch biomass (Fig. 6*b*); however, when considered together in a multiple regression analysis, only predator biomass loaded as a significant explanatory variable for the MI (multiple regression, multiple $R^2 = 0.90$, $p < 0.001$). Perch biomass itself did not add to the explanatory power of this relationship.

Biological responses to lake characteristics

Principal component analysis was used to identify the correlation structure among physical and chemical variables in the study lakes (Table 6). The analysis revealed three significant factors describing almost 80% of the total variability in lake characteristics. The first factor, which explained 37% of the variation, included measures of acidity and lake productivity such as pH, P, Ca, and DOC. An additional 28% of the variation was described by aqueous metal contamination of Ni and Cu (the second factor). The third factor, mostly explained by lake maximum depth, contributed a further 14% of the variation. The acidity and lake productivity factor (factor 1) was positively correlated with perch biomass and negatively correlated with the total fish community biomass, the perch morphological index, and female condition (Table 7). Predator biomass, fish community biomass, male and female condition, female ovary weight, and NDPK activity were positively correlated with the metal contamination factor (factor 2). No biological variables were correlated with factor 3 (Table 7).

Discussion

Community structure

We interpret the dramatic decrease (69%) in yellow perch biomass with increasing total predator biomass as a direct lethal effect of the predators on prey populations, while the indirect effect of the presence of the colonizing predator is thought to generate most of the other life history changes observed in this study. Northern pike and walleye have been documented to feed on yellow perch (Sammons et al. 1994; Kaufman et al. 2006), and yellow perch were found in the summer diet of smallmouth bass from the study lakes (K. Lippert, unpublished data). Perch populations in Sudbury area lakes are known to decline rapidly with the introduction or the recovery of a remnant population of a predator such as lake trout (Gunn et al. 1988, 1990; Gunn and Keller 1990).

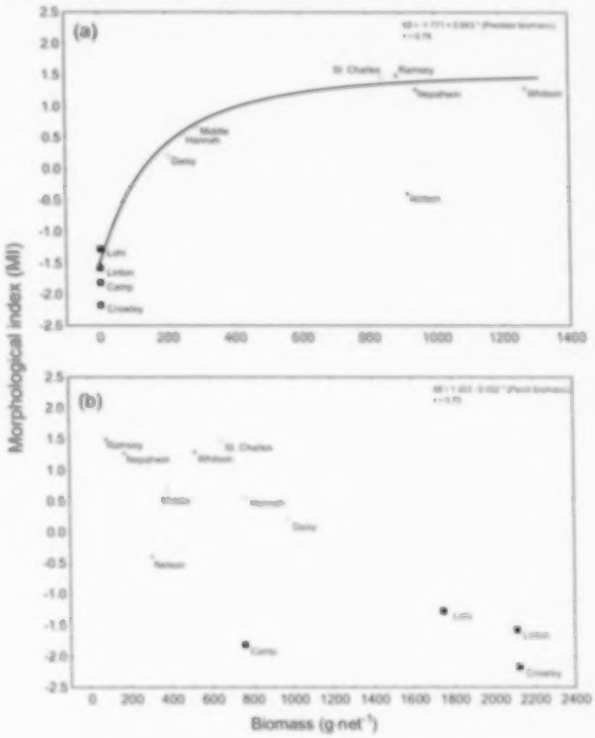

Fig. 6. Relationship between yellow perch body shape (morphological index (MI)) and (*a*) total predator biomass (g·net^{-1}) and (*b*) total yellow perch biomass (g·net^{-1}) among predator regimes: lakes with no predators (■), lakes with recently invaded predators (◇), and lakes with established predators (+).

Summer diet shifts

The differences in sizes at which perch make the ontogenetic shifts from zooplankton to benthic macroinvertebrates are an important finding in this study. Perch in lakes with recently invaded predators appeared to be forced onto benthic resources at approximately half the size of perch from lakes with no predators. Such behavioral changes in prey communities in response to predators have been shown to operate over short time scales (He and Kitchell 1990). Another possible cause for the early shift to benthos could be limitations in the composition and size structure of the offshore zooplankton community. Zooplankton communities were not assessed as part of this study, but it seems unlikely that zooplankton availability would be limited in the recently in-

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Table 6. Summary of the factor loadings, eigenvalues, and percent variation explained for principal component analysis using physical and chemical lake characteristics.

Environmental (lake) variables	Factor 1	Factor 2	Factor 3
Surface area (ha)*	–0.169051	0.685943	0.436055
Maximum depth (m)*	0.499700	0.335518	0.652927
Mean depth (m)	0.645425	0.380004	–0.380004
Secchi depth (m)*	0.493481	–0.297686	–0.297686
pH	–0.847744	0.132919	0.132919
Cu ($\mu g \cdot L^{-1}$)*	–0.414682	–0.842673	–0.061108
Ni ($\mu g \cdot L^{-1}$)	–0.329173	–0.885439	0.114947
P ($\mu g \cdot L^{-1}$)	–0.756492	0.471425	–0.029749
Ca ($mg \cdot L^{-1}$)*	–0.882382	–0.037687	0.357632
DOC ($mg \cdot L^{-1}$)*	–0.670851	0.529041	–0.321756
Eigenvalue (% variation explained)	3.738 (37.4%)	2.831 (28.3%)	1.366 (13.7%)

Note: DOC, dissolved organic carbon.
*Data logarithmically transformed.

Table 7. Pearson correlations (sample size in parenthesis) for yellow perch biological variables and factor scores.

Biological variable	Factor 1	Factor 2	Factor 3
Perch biomass ($g \cdot net^{-1}$)	**0.53** (18)	–0.41 (18)	–0.34 (18)
Predator biomass ($g \cdot net^{-1}$)	–0.22 (12)	**0.63** (12)	–0.09 (12)
Total biomass ($g \cdot net^{-1}$)	**–0.52** (18)	**0.52** (18)	–0.08 (18)
Size shift from zooplankton (mm)	0.34 (14)	0.16 (14)	0.03 (14)
Size shift to benthos (mm)	0.53 (9)	0.47 (9)	–0.18 (9)
Size shift to fish (mm)	0.05 (12)	–0.51 (12)	–0.39 (12)
Egg lipid content ($mg \cdot egg^{-1}$)	0.55 (8)	0.58 (8)	0.15 (8)
Morphological index	**–0.78** (12)	–0.03 (12)	0.44 (12)
Male round weight (g)	–0.46 (18)	**0.61** (18)	–0.23 (18)
Female round weight (g)	**–0.51** (18)	**0.65** (18)	–0.11 (18)
Male testes weight (g)	0.12 (18)	–0.05 (18)	–0.24 (18)
Female ovary weight (g)	–0.39 (18)	**0.48** (18)	0.04 (18)
LDH (IU)	0.21 (14)	–0.10 (14)	–0.18 (14)
NDPK (IU)	–0.34 (14)	**0.65** (14)	–0.23 (14)
CS (IU)	0.37 (14)	0.38 (14)	0.35 (14)
Male length-at-YOY (mm)	0.08 (16)	–0.08 (16)	0.34 (16)
Female length-at-YOY (mm)	0.16 (16)	–0.03 (16)	0.38 (16)
Male length-at-age 1 (mm)	–0.23 (18)	0.13 (18)	0.19 (18)
Female length-at-age 1 (mm)	–0.19 (18)	–0.04 (18)	0.13 (18)
Male length-at-age 2 (mm)	–0.11 (16)	0.04 (16)	–0.09 (16)
Female length-at-age 2 (mm)	–0.11 (17)	0.01 (17)	0.10 (17)

Note: Significant correlations ($P < 0.05$) are noted in bold. YOY, young of the year; NDPK, nucleoside diphosphate kinase; LDH, lactate dehydrogenase; CS, citrate synthase; IU, international units.

vaded lakes given the large reduction in the biomass of perch observed.

Perch from lakes with well-established predators made the shift onto benthos at a similar size to perch from lakes with no predators. However, fish species richness was usually much higher in the lakes with established predators, suggesting that alternate forage fish prey items were available in this lake set. Alternate prey species such as cisco (*Coregonus artedi*) have been shown to buffer perch behavioral changes associated with walleye predation from similar lakes of the Sudbury region (Kaufman et al. 2006). Therefore, the size at which the ontogenetic shift was made onto benthos may have been restored in lakes with established predators because of the buffering capacity of other forage fish species.

Reproductive investment

Males had smaller testes in lakes with predators, indicating that less energy was allocated towards reproduction when predators were present. If perch are confining themselves to less optimal habitats when predators are present, decreased energy acquisition could limit gonadal development in males. However, this trend was not observed in females, suggesting that confinement in refuge areas may not be the explanation. An alternate hypothesis is that variation

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in testes mass may be correlated with perch biomass rather than predator biomass and that the males are responding to decreased intraspecific competition for females when predators have reduced overall densities. Because perch are group spawners, sperm competition would increase in lakes with higher perch biomass (Parker 1982). Therefore, males may invest more in reproduction when the density of competing males is high in the absence of predators.

Female perch in lakes with recently invaded predators showed a significantly lower egg lipid content than in lakes without predators. Egg lipids are an important energy supply for hatched larvae before they initiate feeding. Greater egg lipid content available for the hatched larvae can promote an early start to growth and would likely influence the length achieved at the end of their first growing season (Ware 1975; Johnston 1997). Where offspring habitat quality is low, the results of other studies suggest that females would invest in larger eggs with higher lipid content (Lack 1954; Parker and Begon 1986; Reznick and Yang 1993). However, in our study, females showed an opposite response and produced eggs with low lipid content when challenged by the arrival of an invading predator. This finding, as well as the observation that perch switched to benthic invertebrates at a much smaller size in these early invasion lakes, suggest that somewhat unknown but particularly stressful conditions might exist in these early invasion lakes.

Growth patterns across the predation gradient

Relatively rapid growth, indicated by body mass and NDPK activity, in yellow perch from lakes with established predators appeared to be an indication of indirect trait-mediated effect of predation in these study lakes. Predation may affect prey populations by reducing intraspecific competition, by altering activity or distribution as a behavioral modification to decrease mortality risk, or both (Werner et al. 1983). Reduced activity interpreted as a trait-mediated indirect interaction could increase the amount of energy directed towards growth and (or) reproduction (Peacor and Werner 2001). Size-selective predation may also select for fast-growing phenotypes that rapidly move through vulnerable size ranges of prey (reviewed by Colby et al. 1987; Taborsky et al. 2003). Selection for prey with a heavier mass per unit length may have the same protective effect when the predator is gape-limited.

The observed poor growth of YOY perch in the recently invaded predator lakes continues the pattern observed above, further indicating particularly stressful conditions in these lakes. Two alternate explanations for this finding are that the observed lower egg lipid content reduced growth potential for YOY perch in the lakes and (or) that limited food availability reduced early growth. Age-1 mean length did not differ among the predation regimes, suggesting that the trait-mediated effect of predation on length seen in their first summer of growth was no longer present after their second year of growth. The explanation appears to be that only those individuals with good growth potential could survive the first year of predation or overwinter starvation period. By age 2, females were larger than males, a trend common in perch populations (Scott and Crossman 1973), and this result was consistent across the predator regime. Therefore, after surviving their first year, there were no detectable effects of predators on perch length for a given age-1 or age-2 perch across the predation gradient.

Swimming activity

There was no observed effect of predation on perch swimming behavior based on LDH and CS muscle tissue activity rates. Studies using Trinidadian guppies (*Poecilia reticula*) have shown that burst (estimated by maximum velocity) and aerobic swimming performances (estimated by oxygen consumption rates and CS activity) did not differ significantly between populations with low- and high-predation pressures (Odell et al. 2003; Chappell and Odell 2004). In addition, LDH activity rates were found to be lower in populations with high-predation pressures (Odell et al. 2003). Therefore, factors other than aerobic and anaerobic swimming activities may reduce the risk of predation to perch in these study lakes.

Body shape changes in response to predation

The morphological analysis showed that perch had pelagic body forms in predator-free lakes and littoral body forms in lakes with recently invaded and established predators, a pattern consistent with earlier research (Proulx and Magnan 2002; Svanbäck and Eklöv 2002; Olsson and Eklöv 2005). Dimorphic body forms of Eurasian perch are well documented in lake communities containing predators such as pike, roach (*Rutilus rutilus*), and vendace (*Coregonus albula*) (Svanbäck and Eklöv 2002, 2003). Different phenotypes have been shown to be differentially selected for under various predator regimes (Reimchen 1994; Walker 1997).

In the absence of predators in our lakes, perch had a relatively thin body shape, a more posterior insertion of the spiny dorsal fin, an upwards-pointing snout, and a longer and more slender caudal peduncle area compared with perch from lakes with predators. In lakes with predators present, a shift was observed in the overall body morphology showing increased similarities to the typical littoral form. In lakes with recently introduced predators, the typical shape observed was a thickened body with a short and wide caudal peduncle region, slight anterior migration of the dorsal spiny fin, and a downwards-pointing snout. Increased body thickness in crucian carp (*Carassius carassius*) in response to chemical cues from northern pike has been considered a defensive response to predation because deeper-bodied prey are more difficult for predators to handle and swallow (Hambright 1991; Brönmark and Miner 1992; Nilsson et al. 1995). If this were also true in perch, it would benefit small perch, which are susceptible to predation, to redirect energy towards increased body thickness to decrease vulnerability to gape-limited predators.

Eurasian perch show two distinct morphologies adapted for foraging in littoral and pelagic habitats. Presumably, these morphological differences would be accompanied by activity differences. In our study, we observed morphological differences among yellow perch from different lakes but found no difference in swimming activity as estimated by metabolic enzyme capacities of LDH and CS. There are three possible explanations for the findings: (*i*) changes in body shape across the predator regimes were not large enough to elicit differences in swimming activity across a predation gradient of this magnitude, (*ii*) the morphological

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changes increased swimming efficiency, allowing tissue enzyme activity to remain the same, and (or) (*iii*) phenotypic changes in shape occur faster than phenotypic changes in physiology.

The anterior migration of the spiny dorsal fin may represent a defense mechanism. A greater body depth could be achieved when spines are erected in this body configuration, presenting a defense against a gape-limited predator. The erection of the dorsal spine has been shown to be the first observed response of perch to various visual and chemical cues from pike, irrespective of stimulus intensity or duration (Vainikka et al. 2005). Similar antipredator strategies have been reported in YOY perch that exhibited an increased spine length in the presence of northern pike (Magnhagen and Heibo 2004).

Relation to lake recovery

Improvements in water chemistry and productivity (pH, P, Ca, and DOC) along with declines in metal contamination (Ni and Cu) were correlated with increased fish community biomass in the Sudbury lakes. Yellow perch biomass was negatively correlated with improvements in water chemistry and productivity and unrelated to metal contamination. Predator biomass increased as metal contamination (Ni and Cu) declined but was unrelated to water chemistry and productivity. Few of the yellow perch biological parameters were correlated with factors related to improvements in water quality or metal contamination. Yellow perch shape variation was related to water chemistry and productivity concurrent with decreases in yellow perch biomass (but not overall fish community biomass). Lakes above pH 6.5 appeared to have environmental characteristics amenable to the establishment of predatory fish species. Yellow perch biomass was significantly lower in lakes with predators. The addition of predators to the fish community also triggered a change in yellow perch shape. Although we cannot negate the fact that environmental variables do play an important role in shaping community and perch phenotypes, we found that predator biomass was still the best predictor for these variables.

The elevated metal levels in the water and sediments of Sudbury lakes are, and will remain, long-term concerns (estimates of several centuries to reach background levels) in these industrially damaged lakes (Nriagu et al. 1998). From a physiological or toxicological perspective, there is little evidence that perch, a metal-tolerant species, are severely impacted by the current metal levels (Giguère et al. 2005; Pyle et al. 2005). However, it is well known that many metal-sensitive benthic invertebrate species are scarce or absent from these lakes (Borgmann et al. 2001; Iles and Rasmussen 2005), and reduced benthic availability may be a confounding factor affecting perch bioenergetics in such ecosystems.

Future studies are necessary to fully explain the repercussions of fish predator introductions in recovering lakes. Experiments involving the introduction of predators into prey-only lakes are needed to test many of the predator–prey relationships observed in this study. Detailed studies of benthic invertebrate communities, before and after predator introductions, are also needed to determine whether food limitations are severely impacting prey species in these industrially stressed lakes. For example, do predators create "trophic bottlenecks" by forcing prey into impoverished littoral areas?

Acknowledgements

We thank the Ontario Ministry of Natural Resources (OMNR) for providing a field laboratory, the Ontario Ministry of the Environment for providing water quality data, and the Cooperative Freshwater Ecology Unit staff for their assistance in the field. Lipid extractions were funded by Nigel Lester (OMNR) and equipment and supplies were provided by Tom Johnston (OMNR). This work was supported by the Natural Sciences and Engineering Research Council of Canada. We thank Keith Somers, Erika Genrich, and Charles Ramcharan for statistical advice and Nick Collins, Tom Johnston, and the journal referees for their valuable comments on an earlier draft of this manuscript.

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Numerical changes in stream-resident brown trout (*Salmo trutta*): uncovering the roles of density-dependent and density-independent factors across space and time

Javier Lobón-Cerviá

Abstract: The objective was to uncover the roles of environmental, density-independent, and intrinsic, density-dependent factors as drivers of population size in stream-resident brown trout (*Salmo trutta*) across space and time. The study was based on census data and environmental variables collected every fourth month over 1986–2006 at 12 sites selected along four Rio Esva tributaries (northwestern Spain). Density-dependent hypotheses involving the operation of current and lagged densities on recruitment and subsequent spawner density were analyzed at three increasingly larger geographical scales (site, stream, and river drainage). No evidence supported these hypotheses, suggesting an overall lack of negative feedbacks among year classes. Stream discharge in March when trout emerge and site depth explained substantial amounts of spatiotemporal variation in recruitment. The effects of these two factors on recruitment were sufficiently strong to be detected in year-class mortality and subsequent spawner density. Discharge and site depth determine the amount of spatial habitat suitable for sized individuals during their lifetime and, hence, the maximum number of individuals across space and time. Recruitment dependence, as a delayed density-dependent factor, adjusts the numbers in excess to the numbers established by spatial habitat. These findings differ markedly from the prediction of the most recent theories of population dynamics and are discussed in this context.

Résumé : L'objectif de cette recherche est de découvrir les rôles des facteurs environnementaux indépendants de la densité et des facteurs intrinsèques dépendants de la densité comme variables explicatives de la variation de la taille des populations dans le temps et dans l'espace chez des truites brunes (*Salmo trutta*) résidantes en eau courante. L'étude se base sur des données d'inventaire et des mesures de variables environnementales obtenues à tous les quatrièmes mois en 1986–2006 à 12 sites le long de quatre tributaires du Rio Esva (nord-ouest de l'Espagne). Des hypothèses de densité dépendance impliquant le rôle du courant et des densités déphasées sur le recrutement et la densité subséquente des reproducteurs ont été analysées à des échelles géographiques progressivement plus grandes (site, cours d'eau et bassin versant). Aucune donnée n'appuie ces hypothèses, ce qui laisse croire à l'absence globale de rétroaction négative entre les classes d'âge. Le débit du cours d'eau en mars lors de l'émergence des truites, ainsi que la profondeur du site, expliquent une partie importante de la variation spatio-temporelle du recrutement. Les effets de ces deux facteurs sur le recrutement sont suffisamment importants pour être décelés dans la mortalité des différentes classes d'âge et dans la densité subséquente des reproducteurs. Le débit et la profondeur du site déterminent l'étendue de l'habitat adéquat pour les individus en fonction de leur taille au cours de leur vie et, par conséquent, le nombre maximum d'individus dans l'espace et dans le temps. La dépendance du recrutement, comme facteur de densité dépendance retardé, ajuste les densités en surplus des nombres fixés par l'habitat spatial. Ces résultats diffèrent considérablement des prédictions des théories les plus récentes sur la dynamique des populations et ils sont discutés dans ce contexte.

[Traduit par la Rédaction]

Introduction

Animal populations are dynamic systems in which state variables such as density or age and size distributions fluctuate over time and space. Elucidation of the mechanisms underlying size, persistence, and stability is a central issue in ecological research and critical for the management decisions for exploited populations. Despite the long-standing interest in identifying the rules underlying such variations, few studies have yet identified unequivocally the factors and mechanisms responsible for numerical fluctuations, persistence, and resilience of animal populations across scales of space and time (Ranta et al. 1998; Turchin 2003).

Two opposing and often contentious theories have attempted to elucidate those mechanisms. On the one hand, the endogenous regulation theory formulated by Nicholson (1933, 1957) relies on the operation of negative feedbacks through compensatory density-dependent factors expected to

Received 7 November 2006. Accepted 4 June 2007. Published on the NRC Research Press Web site at cjfas.nrc.ca on 10 October 2007. J19650

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 doi:10.1139/F07-111 © 2007 NRC Canada

occur when individuals compete for limited resources. Such endogenous regulation requires population equilibrium as a long-term stationary probability distribution of population densities (Dennis and Taper 1994), implying that populations fluctuate around mean densities with bounded variances (Royama 1977) and return to equilibrium following any departure (Turchin 1995). In contrast, Andrewartha and Birch (1954) focused on density-independent factors related to habitat quality and variability and argued that there is no equilibrium around which population densities fluctuate. Such nonequilibrium populations can be described as statistically random walks with variances increasing over time (Murdoch 1994).

Density dependence is assumed to be the only process capable of generating stability and resilience (Murdoch 1994) and has been the major focus of population ecology research during the last decades (Coulson et al. 2004). Alternative proposals to reconcile the two theories have been recurrently posed (Huffaker and Messenger 1964; Dempster 1983; Strong 1986). The outline of an emerging consensus acknowledged that on a density-dependent background, the interaction between density-dependent and density-independent factors may not exclude each other but their operational levels would differ across scales of space and time (Hixon et al. 2002; Boyce et al. 2006). This consensus recalls the context-dependent hypothesis (Haldane 1953, 1956) in which the operation of density-dependent factors would predominate in favorable environments at high densities and density-independent factors would predominate in unfavorable environments at low population levels.

The brown trout (*Salmo trutta*) is a polymorphic species that shows a wide spectrum of life history patterns within (Cucherousset et al. 2005) and among (Crisp 2000) populations. Stream-living populations combine variability in life history with the environmental complexity that typifies stream habitats across scales of space (stream gradients across climatic regions) and time (seasonal and annual climatic conditions) (Pringle et al. 1988). Consequently, these populations are excellent targets for the investigation of population regulation. Intensive research throughout its native European distributional range (Mann et al. 1989; Maisse and Beglinière 1999; Crisp 2000) and in regions where it has been introduced (Hayes 1995; Carline 2006) exemplify the controversy on the relative importance of density-dependent and density-independent factors as drivers of population size, stability, and persistence.

Long-term studies in UK streams provided support for the occurrence of endogenous feedback loops through the operation of density-dependent mortality on the youngest juveniles (Elliott 1985) or through delayed density dependence on the subsequent density of spawners (Elliott and Hurley 1998). Similar studies in Spain (Lobón-Cerviá and Rincón 2004), France (Cattanéo et al. 2002), and Denmark (Lobón-Cerviá and Mortensen 2005) emphasized environmental, density-independent drivers of population size related to the stream discharge conditions operating during the youngest life stages. The context-dependent hypothesis in which density-dependent processes predominate in favorable habitats and density-independent processes predominate in unfavorable habitats found support in observational (Elliott 1987) and ecological modeling studies (Daufresne and Renault 2006). Also, the strength of recruitment dependence has been suggested to operate as a compensatory driver of the density of juveniles and older life stages (Rasmussen 1986; Elliott 1996).

Such variability in results suggests the need for further studies aimed at identifying the contexts or scenarios under which the suites of factors and mechanisms of population regulation operate. The present large-scale study attempts to uncover the roles of density-dependent and density-independent factors as determinants of temporal and spatial variations in the numerical abundance of stream-resident brown trout across the complex environmental heterogeneity that typifies Cantabrian river drainages (northwestern Spain). A prior study on one of these streams offered compelling evidence of environmentally driven population size (Lobón-Cerviá and Rincón 2004). The present study addressed a substantially larger spatial scale to include a variety of replicated habitats (Ray and Hasting 1997) of variable quality in contrasting streams of the Río Esva drainage in which brown trout showed a variety of densities ranging from persistently high to persistently low over prolonged time periods.

Specifically, I attempted to elucidate whether the role of density-dependent and density-independent factors, as drivers of population size, was related to the habitat quality at the scale of a site vis-à-vis population traits, or alternatively, whether these suites of factors operated at broader geographical scales across the environmental heterogeneity of the whole-river drainage. The following points were explored to address this issue: the relative importance of the parental density (*i*) for annual recruitment and (*ii*) for the subsequent abundance of female spawner density; (*iii*) the relative importance of recruitment as a driver of the subsequent spawner density; (*iv*) spatial synchrony in major population traits across the patchwork of sites; and (*v*) whether, what, when, and where density-independent environmental factors operated on population density under no density-dependent regulatory mechanisms. I explored the occurrence of density dependence and density independence over prolonged time scales encompassing the years 1986–2006 across increasingly larger spatial scales, namely, at the scale of a stream site, at the scale of a single stream, and at the scale of whole-river drainage.

Materials and methods

Study area

Río Esva is one of the numerous coastal drainages of the 700 km long Cantabrian corridor of northwestern Spain, the waters of which flow north to its mouth in the Cantabrian Sea. Río Esva is a medium-sized drainage (~500 km^2) that contains a complex net of low altitude streams of variable size and physiography flowing over quartzite bedrock. From the source at about 700 m above sea level (asl), well-oxygenated, rapid waters flow across rugged, deep, forested valleys fed by rainfall and runoff. Within the rather continuous rainy conditions (~1500 mm·year^{-1}), increased spring and autumn rainfall may accentuate discharge variations. Mild winters and summer air temperatures that seldom reach 30 °C result in mean annual stream temperatures around 11–14 °C, near the optimum of 13 °C for trout growth (Elliott 1994; Lobón-Cerviá and Rincón 1998).

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Table 1. Major characteristics of the 12 sites of Rio Chaballos, La Viella, Castañedo, and Choudral (Rio Esva drainage, Asturias, northwestern Spain) examined in this study.

Site	Length (m)	Width (m)	Depth (cm)	Area (m^2)	Velocity ($m \cdot s^{-1}$)	Canopy (%)
Rio Chaballos						
Ch1	75	2.8	20	240	0.25	10
Ch2	80	3.3	26	250	0.36	15
Ch3	70	3.8	21	220	0.31	20
Ch4	75	3.9	27	370	0.18	80
La Viella						
V5	70	3.4	18	260	0.20	85
V6	65	2.7	22	160	0.23	60
V7	70	3.2	19	190	0.30	30
Castañedo						
C8	65	2.9	23	150	0.13	40
C9	65	2.9	26	180	0.16	40
C10	75	4.0	25	320	0.21	70
Choudral						
Cho11	65	2.0	15	150	0.16	92
Cho12	70	2.4	16	200	0.15	95

Note: All values are means quantified from around 500 bathymetric maps drawn after every census conducted at base-flow conditions during the years 1986–2006.

Along the streams, the environmental complexity was enhanced by the occurrence of patches of grass meadows alternating with riparian forest composed of native species (*Salix alba* L., *Alnus glutinosa* (L.) Gaertner, *Quercus robur* L., *Pinus pinaster* Aiton, and *Betula alba* L.) mixed with a few aliens (*Castanea sativa* Miller, *Corylus avellana* L., *Pinus radiata* D.Don, and *Eucalyptus globulus* Labill). Thus, stream reaches varied from fully covered by overhanging vegetation (canopy) to completely open, unshaded sites in meadows. These features combine with abundant reproductive and feeding habitats over gravel-, pebble-, and boulder-dominated streams to offer optimal conditions to meet brown trout requirements (Armstrong et al. 2003). Consistent with these conditions, surveys prior to this study revealed a full occupation of Rio Esva by brown trout. Individuals of all ages thrived along the main stem and in all tributaries from the sources down to the river mouth in the Cantabrian Sea. Juveniles and adult individuals showed substantial variations in abundance across spatial scales, but no stream section even <10 m long lacked individuals (J. Lobón-Cerviá, unpublished data).

Streams and sites were selected to include the environmental heterogeneity and variability of trout densities apparent across the Rio Esva landscape. Samples prior to this study identified stream sections of variable densities where electrofishing techniques provided highly accurate density estimates (Lobón-Cerviá 1991, 2003). Accordingly, the study sites were selected to include a within-site run–riffle–pool succession, the variation in channel size, structure, and canopy, from fully shaded to totally open sites, and to be of a size to maximize the accuracy of population estimates (length 60–80 m, width 2–10 m, maximum depth < 1.0 m). As a consequence, the 12 study sites were scattered along four subdrainages, two flowing east to west towards the main stem of Rio Esva and the other two flowing west to east. In turn, these four streams are representative of the numerous streams flowing north and south across the Cantabrian corridor. The streams and sites are as follows: Rio Chaballos (four sites, Ch1 to Ch4); Rio La Viella (three sites, V5 to V7); Rio Castañedo (three sites, C8 to C10); and Rio Choudral (two sites, Cho11 and Cho12). Major characteristics are summarized in Table 1 and Lobón-Cerviá (2004).

Data collection and the brown trout populations

As data collection and sampling procedures have been described elsewhere (Lobón-Cerviá 2003, 2005), only features relevant for this study are described. The number of individuals was quantified by electrofishing, applying the three-pass removal method (Lobón-Cerviá 1991). Monitoring censuses, at least three times per year (January, May, and September), have continued from 1986 in Rio Chaballos and from 1989 in the other three streams. However, Rio Castañedo was not accessible from 2003 onwards and one of the two sites in Rio Choudral was not accessible from early 2006. All censuses were conducted under base-flow conditions. After fish sampling, detailed bathymetric maps were drawn and a variety of environmental parameters were recorded for each site. Daily rainfall and discharge conditions were recorded by the Trevias gauging station located in the main stem of Rio Esva at mid-distance from studied streams. It was assumed that daily variations recorded at this station reflected concurrent variations throughout the river drainage.

A simultaneous 10-year study on the reproductive ecology of brown trout in the same streams showed that spawning occurred from the end of November and emergence occurred by mid-March. Thus, the birth date was defined as 15 March. This study also revealed a constant 1:1 sex ratio across spatiotemporal scales and a strong tendency to semelparity, in which all 21-month-old females >11.5 cm spawned successfully every year, but a few females survived to spawn twice (Lobón-Cerviá et al. 1997).

During the years 1988–1994, extensive mark–recapture procedures in Rio Chaballos and La Viella were combined with the operation of two traps located at the mouth of the

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two streams to collect fish moving downstream. The results revealed low mobility and high site fidelity (Lobón-Cerviá 2000, 2003), with some 70% of the juveniles marked at any given site and year being recaptured at the same site 8–12 months later. No trout tagged in a site was ever collected in any other site of the stream and no fish tagged in any site was ever captured in the downstream traps, even if the nearest tagging sites were at <400 m and <200 m upstream from the traps in Rios Chaballos and La Viella, respectively. Moreover, the trout collected in the traps downstream yielded figures of 1 trout·month^{-1} with little among-month or among-year variation.

Overall, the age, size structure, and density observed across sites and years in these Rio Esva tributaries typify resident populations in streams flowing along the Cantabrian corridor of northwestern Spain (García and Braña 1988; Reyes-Gavilán et al. 1995).

Hypothesis testing and data analysis

A core density-dependent hypothesis defines the role of the reproductive component of the population for the abundance of the subsequent offspring. In fishery ecology, this relationship is known as a stock–recruitment relationship (Ricker 1954). In populations where the abundance of the youngest juveniles is determined by density-independent processes, recruitment may show no relationship with parental density or may increase proportionally with increasing parental density. In populations where the abundance of the youngest juveniles is determined by density-dependent processes, recruitment may increase potentially with no limit as the parental density increases and may become asymptotic or dome-shaped at high parental densities under compensatory regulation. Therefore, the operation of density dependence is necessary but not sufficient evidence for recruitment regulation. The latter requires compensatory adjustments in the form of constant or declined recruitment magnitudes at high parental densities.

The suite of factors operating on the abundance of post-recruitment stages may be sensitive to habitat quality and differ across life stages and populations. However, the recognized importance of recruitment processes not only suggests that the annual magnitudes of recruitment would be reflected in the age structure of the population (Caley et al. 1996), but also that the strength of recruitment may operate as a compensatory factor, adjusting the subsequent parental density or the reproductive potential (Gee et al. 1978; Gardiner and Shackley 1991). This being the case, population regulation through recruitment dependence would cause asymptotic or dome-shaped curves of adult density with increased recruitment.

The rates of population change may be modulated by the effects of the current density or by delayed density dependence (Turchin 1990). Therefore, the potential effects of both current and lagged densities were explored through three density-dependent hypotheses: (*i*) annual recruitment is determined by the parental density; (*ii*) the subsequent abundance of females surviving to spawn 2 years later is determined by the original parental density; and (*iii*) the subsequent abundance of female spawners is determined by recruitment dependence. The three year-class traits required to test these hypotheses were considered to be: (*i*) parental density (ST, individuals·m^{-2}), the number of females prior to spawning; (*ii*) recruitment, the number of age-0+ juveniles in May when they were 2 months old (R, individuals·m^{-2}); and (*iii*) the subsequent female spawner density (SA, individuals·m^{-2}), the abundance of females about to spawn 2 years later.

These three density-dependent hypotheses were tested by exploring the suitability of a general, domed-shaped Ricker model (Ricker 1954) in the following form:

$$(1) \qquad R = A \cdot \mathrm{ST} \cdot e^{-B \cdot \mathrm{ST}}$$

In this compensatory model, the variations in annual recruitment R are first accounted for by a density-independent process (the first term of the equation, A·ST) and then by the operation of density dependence (the second term of the equation, $e^{-B \cdot \mathrm{ST}}$). To test the other two density-dependent hypotheses, R is replaced by SA and ST by R.

Bellows (1981) established that when fitting a density-dependent model to data, the operation of density dependence can be assumed if and only if the following hypotheses can be rejected: (1) recruitment varies around a mean value so that R_1 = mean R and (2) recruitment depends on the parental density showing a constant proportional survival where mortality is caused by density-independent factors, i.e., $R_2 = A \cdot \mathrm{ST}$. These two hypotheses are tested by fitting the density-dependent model to data by unweighted least squares iterative algorithms and estimating the coefficient of determination R^2 as

$$R_1^2 = 100[(1 - \text{residual SS})/\text{SS about the mean } R]$$

and

$$R_2^2 = 100[(1 - \text{residual SS})/\text{SS about the line } A \cdot \mathrm{ST}]$$

where SS refers to sum of squares. The significant levels are obtained by the proportion of variance using the F values of the variance ratio (Motulsky and Christopoulos 2004).

The residuals of the stock–recruitment curves were used as indices of survival rates of recruits and of the subsequent spawner. Spatial synchrony, as the concurrent changes in population traits across spatial scales (Liebhold et al. 2004), were explored through Pearson's product moment correlations among pairwise comparisons for the indices of survival rates of recruits and of the subsequent spawners and for year-class mortality rates. Under the repeated-test principle in univariate analysis, there is an inherent risk of alpha inflation. However, Moran (2003) has recently argued that such analysis is appropriate with no Bonferroni corrections. Because my analysis attempts to explore trends in temporal synchrony, I reinforced the results of pairwise correlations examining levels of concordance in the population traits across temporal scales through Kendall's coefficient of concordance (W), where W, without corrections for ties, is obtained as

$$W = [(\Sigma D_i^2 - (\Sigma D_i)^2/n]/[M^2(n^3 - n)/12]$$

where D_i represents ranked densities across years. The significance of this is tested with a Friedman χ^2 of the form

$$\chi^2 = M(n - 1)W$$

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where M is the number of variables (i.e., study sites) and n is the number of data pairs (Zar 1984).

Several functional relationships in this study showed split-line trajectories in which the dependent variable (Y) increased linearly with increased independent variable (X) up to the point at which the dependent variable sharply changed direction and decreased linearly as the independent variable continued to increase. To fit these continuously ascending and descending trajectories, I used the following two-phase regressions:

$$Y = A_1 + B_1X\,(X \leq K) + A_2 + B_2X\,(X > K)$$

For computational purposes, this regression can be rewritten as

$$(2)\qquad Y = A_1 + B_1X\,(X \leq K) + [A_1 + (B_1K + B_2X + XK)]\qquad (X > K)$$

This modification offers the advantage that only one elevation (A_1) and the two slopes (B_1 and B_2) of the ascending and descending portions are to be estimated. The two-phase trajectory is restricted for continuity by $A_1 + B_1K = A_2 + B_2K$ at the breakpoint K, where the trajectory changes direction (Motulsky and Christopoulos 2004).

Based on previous studies (Lobón-Cerviá 2004; Lobón-Cerviá and Rincón 2004), I focused the analysis of relationships between population traits and environmental factors on two major stream variables: year-to-year variations in discharge (Hm^3-month^{-1}) in March when trout emerge and on the spatial variation in site depth (cm). The latter was the mean depth of the sites under base-flow conditions and was quantified on the bathymetric maps averaged across sampling dates over the whole study period.

The selection of sites was essentially a nested design (sites within streams). Therefore, nested analyses of variance (ANOVAs) were used to highlight the relative effects of the site and stream features on relevant population traits. All linear and nonlinear regressions were fitted by quasi-Newton least squares algorithms.

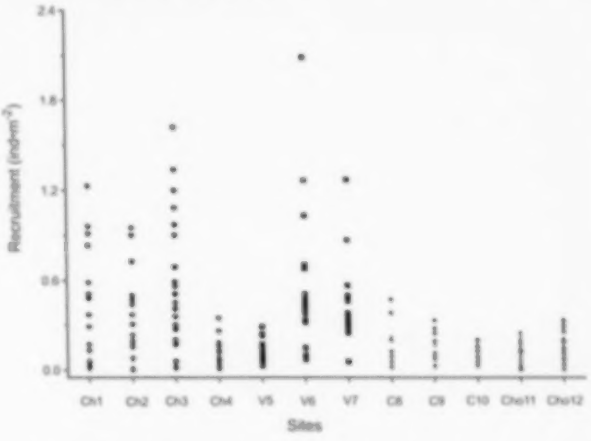
Results

Spatiotemporal variations in recruitment and parental density

Over the study years and sites, the annual recruitment varied 200-fold (Fig. 1), ranging from negligible amounts to markedly high magnitudes (overall range 0.01–2.02 individuals·m^{-2}). A nested ANOVA revealed significant differences among streams ($F_{[3,195]} = 10.85$, $p < 0.001$) and among sites ($F_{[8,195]} = 7.12$, $p < 0.001$). Although in several sites the annual magnitudes varied temporally within narrow ranges, in other sites the ranges of variation were greater and the relative differences between narrow and broad ranges were comparable among nearby sites within a stream and for sites separated by long distances in different streams (Fig. 1).

The rates of temporal change in recruitment measured as $\log(R_{t+1}) - \log(R_t)$ revealed that at all site recruitment values lie uniformly above and below the zero change (Fig. 2), indicating that at a site scale, recruitment over the study period showed no obvious temporal trend. As a consequence, the relative differences among sites in recruitment were considerably greater in years of high recruitment and became obscured in years of low recruitment.

Fig. 1. Variations in annual recruitment (individuals (ind)·m^{-2}) over the years 1986–2006 at the 12 study sites of Rio Chaballos (Ch1–Ch4), La Viella (V5–V7), Castañedo (C8–C10), and Choudral (Cho11–Cho12) tributaries of the Rio Esva drainage (Asturias, northwestern Spain).



The reproductive component of the population measured as the parental density (ST, individuals·m^{-2}) and the subsequent spawner density (SA, individuals·m^{-2}) varied within similar or even greater ranges across space and time (for ST, range = 0.006–0.30 individuals·m^{-2}; for SA, range = 0.001–0.22 individuals·m^{-2}). The among-site differences were smaller than those in recruitment, and unlike recruitment, nested ANOVAs indicated major effects of the site (for ST, $F_{[8,191]} = 3.76$, $p < 0.001$; for SA, $F_{[8,171]} = 0.52$, $p < 0.001$) and nonsignificant effects of the stream (for ST, $F_{[3,191]} = 1.56$, $p = 0.20$; for SA, $F_{[3,171]} = 0.39$, $p < 0.77$).

Spatiotemporal variations in year-class mortality

At all sites, year-class density was remarkably constant over age, indicating a prolonged time period of no mortality. The onset of mortality occurred at a given time after which density declined sharply and continuously until the total disappearance of the year class. Two-phase regressions suitably fitted the successive densities over time for each year class and site. These fits revealed that most year classes showed no detectable postrecruitment mortality over a prolonged time period that varied between 450 and 750 days after emergence depending on the site, implying that the onset of mortality differed by about 300 days among sites. Several year classes did show mortality during this first period, but in all instances, these mortalities were substantially lower than in the second period. Moreover, recruitment of the 1995 and 1997 year classes was extremely low at several sites. The few individuals commencing these year classes remained practically constant in density throughout the lifetime of the year class.

The severe and continuous mortalities of the second period accounted for ≥80% of the total year-class mortality. For the sake of simplicity, these patterns are exemplified by the 1990–1994 year classes at the 12 sites (Fig. 3). Given the predominance of mortality during the second half of the life-

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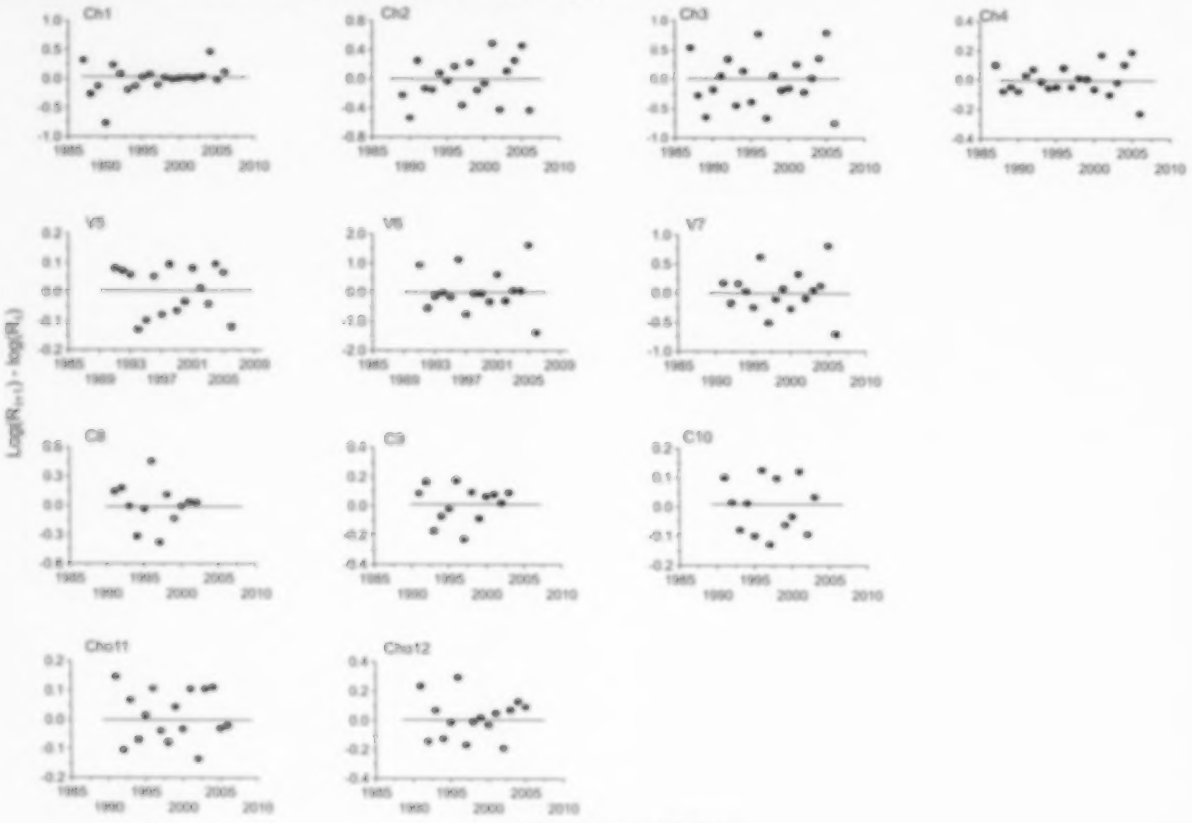
Fig. 2. Rates of change in the annual recruitment $\log(R_{t+1}) - \log(R_t)$ of brown trout over the years 1986–2006 at 12 study sites of Rios Chaballos (Ch1–Ch4), La Viella (V5–V7), Castañedo (C8–C10), and Choudral (Cho11–Cho12) tributaries of Rio Esva (Asturias, northwestern Spain). The lines indicate the zero change $\log(R_{t+1}) = \log(R_t)$.



time, I focused this study on these mortalities, the rates of which were estimated as the slope of the second component of the two-phase regression and were expressed as $Z\text{-day}^{-1} = -100 \times \text{slope}$.

A nested ANOVA for the mortality rates revealed significant effects of site ($F_{[8,177]} = 4.85$, $p < 0.001$) but nonsignificant effects of stream ($F_{[3,177]} = 1.67$, $p = 0.25$). An ANOVA for the within- and among-site effects induced by the year of emergence revealed strong effects of the site ($F_{[11,177]} = 10.0$, $p < 0.001$) and of the year of emergence ($F_{[17,177]} = 9.78$, $p < 0.001$) that explained 27.6% (site), 38.8% (year), and 34.5% (site – year of emergence interaction) of the variance, accounting for practically all variance of mortality (residual variance ~ 0).

An appraisal of the operation of density dependence

Parental density – recruitment relationships

Visual inspections of the scatter plots of annual recruitment versus parental density revealed a great variability in which not only did sites with similar parental densities show markedly different magnitudes of recruits, but also the shape generated by the dispersion of the data differed substantially among sites (Fig. 4).

A fit of the Ricker model to the 204 year classes pooled revealed an extremely weak predictive ability of parental density whereby only 3.2% of the variance in spatiotemporal recruitment was explained by the variations in parental density. Similarly weak fits were obtained for single streams (pooled sites), explaining 17.9% of the variance for Rio Chaballos (78 year classes), 0.2% for Rio La Viella (51 year classes), 0.3% for Rio Castañedo (41 year classes), and 1.3% for Rio Choudral (34 year classes). Fits for each single site separately highlighted a variety of shapes in the relationship between recruitment and parental density (Fig. 4). The variance in recruitment explained by these fits was greater than that for streams and for the whole data set (Table 2*a*). However, except at site Ch1 where 61.7% of the variance in recruitment (20 year classes) was explained nonsignificantly ($p = 0.28$) by the parental density, at all other sites, the effects of the parental density were nonsignificant and only explained between 0.1% and 18.0% of the variance in recruitment.

Hypothesis testing for the basic hypothesis R = mean R for single sites, for streams, and for the whole data set (Table 2*a*) showed that in 6 out of the 17 possible instances, the tests were irrelevant given that the SS obtained by fitting the density-dependent model were greater than those around the

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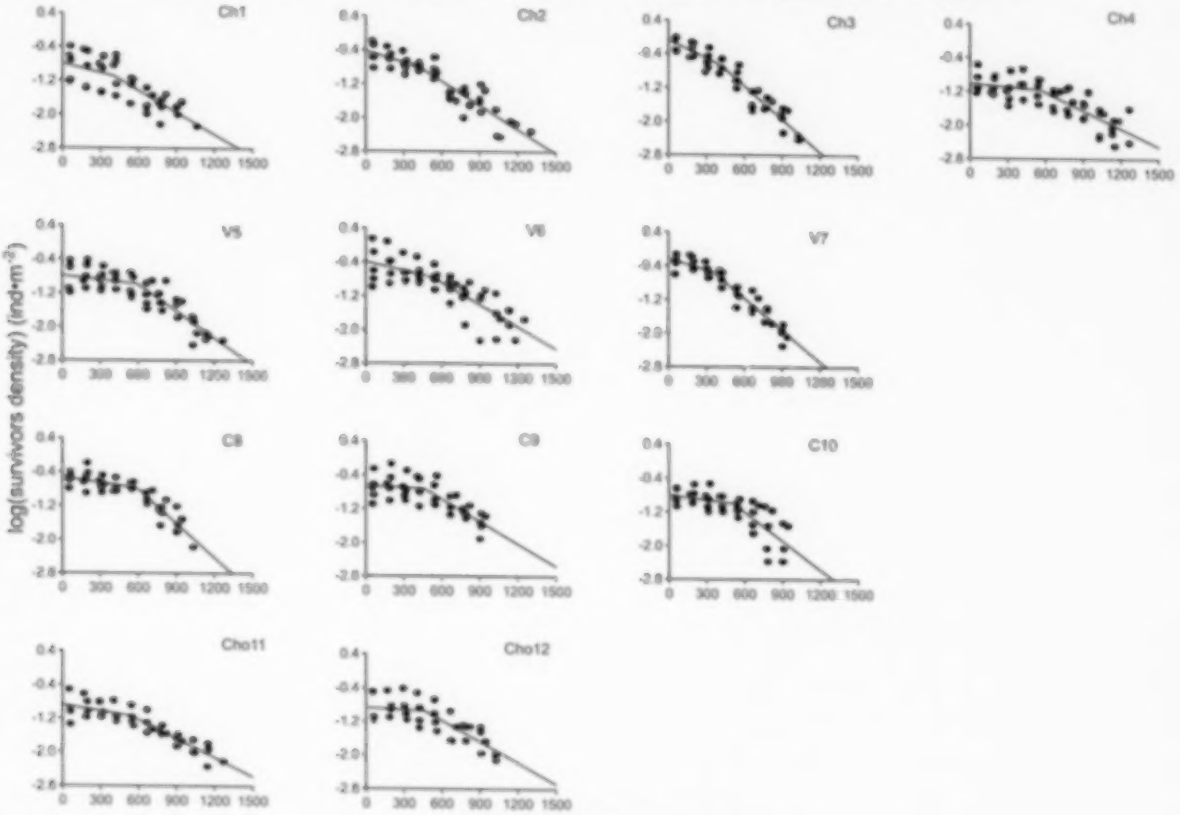
Fig. 3. Two-phase patterns depicted by log-transformed survivors density (individuals (ind)·m^{-2}) over age (days after emergence) as exemplified by the 1990–1994 year classes of brown trout at 12 study sites of Rio Chaballos (Ch1–Ch4), La Viella (V5–V7), Castañedo (C8–C10), and Choudral (Cho11–Cho12) tributaries of Rio Esva (Asturias, northwestern Spain). A prolonged period of no or negligible mortality is followed by a second period of heavy mortality that lasts until the total disappearance of the year classes.



mean recruitment (Table 2*a*). Tests for $R = A \cdot ST$ revealed that although this density-independent model explained less of the variance in recruitment than the density-dependent model, the differences were not significant. At the three spatial scales (site, stream, and drainage), there was no significant evidence of a density-dependent relationship between parental density and recruitment.

Parental density – spawner density relationships

The relationships between parental density and the subsequent spawner density also showed a variety of shapes (Fig. 5). Fits of Ricker model revealed a very weak ability of the parental density to predict the subsequent spawner density either for the whole data set (0.1%, 172 year classes) or for the single streams: Rio Chaballos (2.3%, 70 year classes), La Viella (13.8%, 45 year classes), Castañedo (8.8%, 35 year classes), and Choudral (23.1%, 29 year classes). Fits for each single site were slightly stronger, yet they revealed a weak predictive ability of parental density (Fig. 5).

In all instances, the levels of significance were extremely low to the extent that in no case did the density-dependent model perform better than the density-independent model either for single sites or streams or the whole data set (Table 2*b*).

Recruitment – subsequent spawner density relationships

Visual inspections indicated linear trends between the subsequent spawner density and recruitment at the 12 sites (Fig. 6). Linear trends were so consistent that the analysis of density-dependent model was omitted and I fitted directly linear regressions. These linear regressions underscored strongly significant relationships (Table 3). Between 29.4% (*i.e.*, site Cho12) and 68.1% (i.e., site Ch3) of the temporal variance in the subsequent spawner abundance was explained by variations in recruitment (Table 3). Moreover, these relationships were also significant for single streams and for the whole data set (Table 3), emphasizing the important role of recruitment as a determinant of the subsequent spawner abundance.

In these fits, the elevations of the site-specific linear regressions were not significant in nine out of the 12 sites but were significant for single streams and for the whole data set (Table 3). An analysis of covariance (ANCOVA) for the set of 12 regressions revealed no significant among-site differences in the elevations ($F_{[11,147]} = 0.88$, $p = 0.56$) but highly

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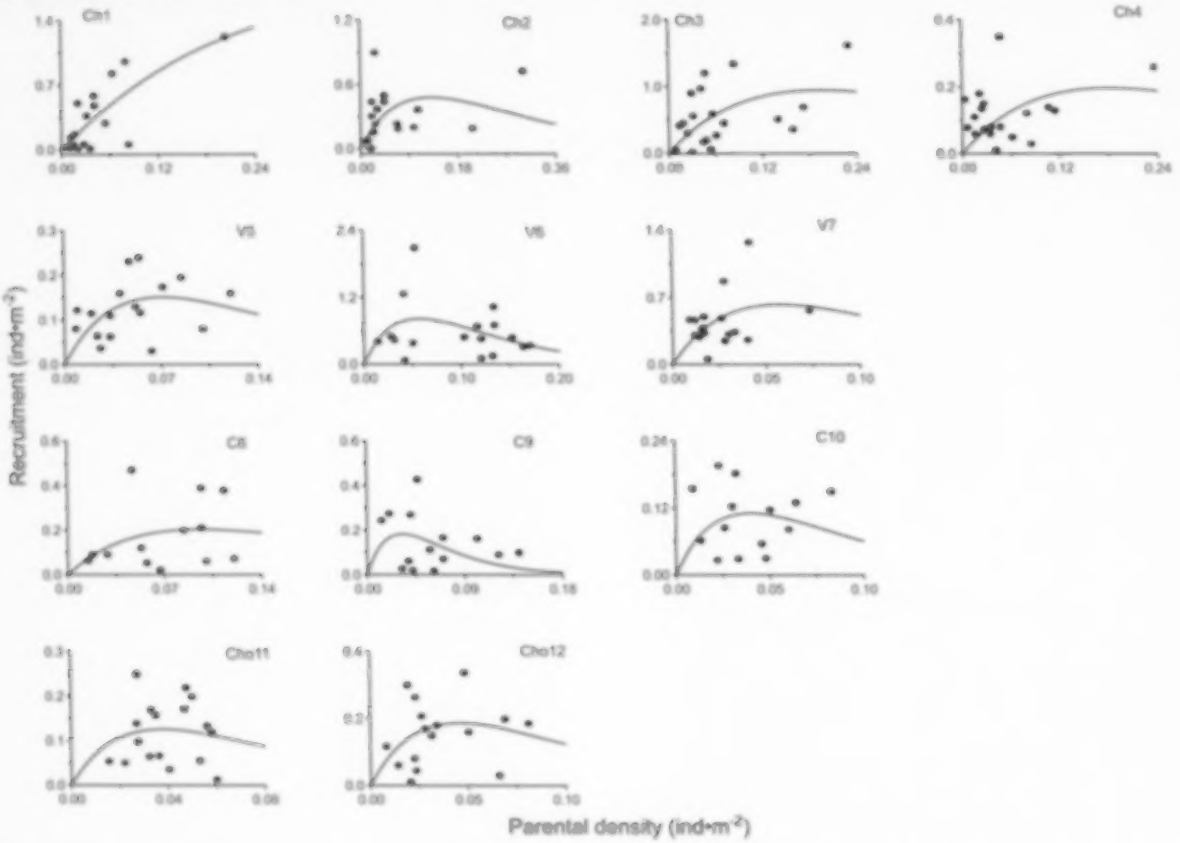
Fig. 4. Relationships between parental density (individuals (ind)·m^{-2}) and recruitment (ind·m^{-2}) of brown trout across year classes at 12 study sites of Rio Chaballos (Ch1–Ch4), La Viella (V5–V7), Castañedo (C8–C10), and Choudral (Cho11–Cho12), with a domed-shaped Ricker model fitted to the data.



significant differences ($F_{[11,147]} = 2.41$, $p = 0.009$) among the slopes, which was strongly consistent with the previously detected among-site differences in the mortality rates.

Effects of recruitment on year-class mortality

The trajectories described by the mortality rates during the second period of the lifetime vs. recruitment for each single site indicated an overall tendency to positive power relationships. Interestingly, a plot for the 164 year classes pooled showed that variations in the mortality rates described a smooth and continuous power trajectory over recruitment. A fit of a common power function was highly significant (Fig. 7), with 54.3% of variance in the mortality rates across space and time explained by variations in recruitment. In this relationship, the rates at which mortality increased with increased recruitment were markedly steeper at low recruitment levels and became shallow at high levels. Thus, small increments in recruitment at sites where trout showed persistently low recruitment (i.e., sites Ch4, V5, and Cho11) brought about steep changes in mortality relative to those sites where trout showed persistently high densities (i.e., Ch3 and V6). This common power relationship emphasized importance of recruitment for the survival of individuals during the second half of the lifetime at all sites through strong effects of delayed density dependence.

Spatial synchrony in the temporal variations of recruitment, mortality, and spawner density

Pairwise correlations among the indices of survival rates of recruits across sites were all positive (Table 4*a*). Among the 66 possible correlations, 53 (80.3%) were significant in the range $0.001 < p < 0.05$; three instances in the range of $0.05 < p < 0.10$, and 10 instances in range $p \geq 0.10$. Moreover, all pairwise correlations among the indices of survival rates of the subsequent spawner density were also positive, and among the 66 possible correlations, 38 instances (58%) were significant in the range of $0.001 < p < 0.05$, five instances were significant in the range of $0.05 < p < 0.10$, and in 23 instances the significance levels were at $p \geq 0.10$ (Table 4*b*). Interestingly, pairwise correlations among the year-class mortality rates across sites were also shown to be positive and revealed high levels of significance (Table 4*c*). Among the 66 possible instances, 40 instances were significant at $0.001 < p < 0.005$, nine instances at $0.05 < p < 0.10$, and 17 instances at $p \geq 0.10$. In the three comparisons, all correlations were positive and the levels of significance were similar among

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Table 2. Tests for current and delayed density-dependent compensatory hypotheses for Río Esva brown trout where the fit of a stock–recruitment model for (*a*) parental density – recruitment relationships $R = A{\cdot}ST{\cdot}e^{-B{\cdot}ST}$ and (*b*) parental density – subsequent spawner density relationship $SA = A{\cdot}ST{\cdot}e^{-B{\cdot}ST}$ are tested against the two basic hypotheses R = mean R and $R = A{\cdot}ST$ and, correspondingly, SA = mean SA and $SA = A{\cdot}ST$.

(*a*) Parental density – recruitment relationship.

		R = mean R			$R = A{\cdot}ST$		
	N	R^2	F	p	R^2	F	p
Río Chaballos	78	20.7	0.4	0.52	13.4	0.2	0.63
Ch1	20	6.2	2.2	0.24	6.8	0.02	0.88
Ch2	18	IR			28.1	0.2	0.69
Ch3	20	IR			12.9	0.1	0.72
Ch4	20	IR			18.2	0.1	0.78
La Viella	51	0.2	0.2	0.63	25.0	0.01	0.99
V5	17	IR			24.5	0.5	0.48
V6	17	13.8	1.0	0.32	43.9	2.4	0.13
V7	17	9.9	0.7	0.41	26.4	1.3	0.26
Castañedo	41	IR			22.1	0.4	0.51
C8	13	8.5	0.2	0.62	9.5	0.2	0.63
C9	14	20.6	0.01	0.94	56.1	0.1	0.71
C10	14	IR			36.5	0.03	0.87
Choudral	34	2.2	0.03	0.96	28.7	1.2	0.28
Cho11	17	6.3	0.04	0.85	26.0	0.5	0.51
Cho12	17	8.0	0.04	0.84	38.3	1.0	0.32
Total	204	10.3	0.12	0.73	6.3	0.05	0.83

(*b*) Parental density – subsequent spawner density relationship.

		SA = mean SA			$SA = A{\cdot}ST$		
	N	R^2	F	p	R^2	F	p
Río Chaballos	66	IR			IR		
Ch1	17	39.5	0.96	0.33	32.4	0.66	0.42
Ch2	15	10.4	0.06	0.81	49.9	1.07	0.31
Ch3	17	5.6	0.006	0.94	14.2	0.08	0.78
Ch4	17	14.3	0.03	0.86	8.6	0.02	0.88
La Viella	42	13.2	0.10	0.75	16.4	0.13	0.72
V5	14	IR			12.0	0.08	0.78
V6	14	1.6	0.002	0.96	39.8	0.97	0.33
V7	14	IR			16.1	0.14	0.71
Castañedo	35	8.9	0.05	0.83	22.1	0.40	0.53
C8	11	IR			8.3	0.04	0.85
C9	12	IR			53.9	0.99	0.33
C10	12	IR			IR		
Choudral	28	25.0	0.13	0.88	46.1	1.36	0.20
Cho11	14	IR			24.6	0.93	0.35
Cho12	14	IR			51.3	1.49	0.23
Total	171	9.1	0.63	0.43	26.5	1.27	0.27

Note: Results are presented for each of the 12 study sites, for single streams (pooled sites) and for the whole data set. N, the number of data pairs; R^2, the variance explained by the density-dependent model over the corresponding single model; F, the F ratio of variance; p, the significance level. Instances where the sum of squares obtained by the fit of the density-dependent model were greater than those obtained by either of the two basic models are considered irrelevant (IR).

nearby sites within a stream and among sites located at a distance in different streams.

Strongly consistent with those results, concordance tests (W) were highly significant for the survival rates of recruits ($\chi^2_{12} = 153.6$, $p < 0.001$), for the survival rates of the subsequent spawner density ($\chi^2_{11} = 77.6$, $p < 0.001$), and for the mortality rates ($\chi^2_{12} = 73.6$, $p < 0.001$). Thus, concordance tests and correlations analyses corroborated each other to offer evidence that the three major year-class traits (recruitment, mortality, and spawner density) were strongly synchronized across temporal scales. Such synchronization was also consistent with the recruitment-dependent mortality and spawner density relationships and enhanced the role of delayed density dependence as a driver of population numbers

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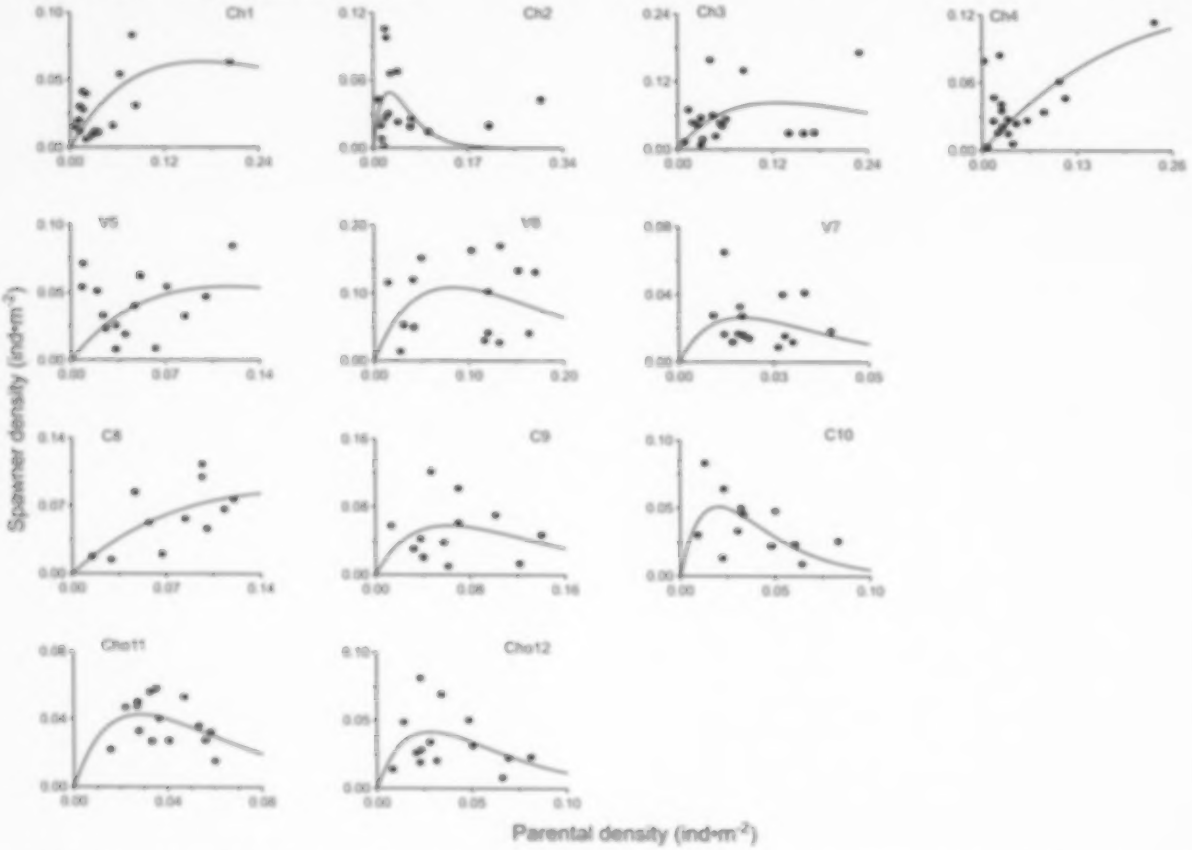
Fig. 5. Relationships between parental density (individuals (ind)·m^{-2}) and subsequent spawner density (ind·m^{-2}) across year classes of brown trout at 12 study sites of Rio Chaballos (Ch1–Ch4), La Viella (V5–V7), Castañedo (C8–C10), and Choudral (Cho11–Cho12), with a dome-shaped Ricker model fitted to data.



during the second half of the lifetime across temporal scales throughout the river drainage.

An appraisal of the operation of environmental factors

Effects of discharge on the temporal variations of recruitment

Visual inspections of the indices of survival rates of recruits against stream discharge in March when trout emerged highlighted similar patterns for the whole data set, for each single stream, and for each site separately. At the 12 single sites (Fig. 8), survival rates were the lowest at minimum discharge and increased linearly with increasing discharge to attain maximum values at average discharge conditions, beyond which these rates decreased linearly with increased discharge (Fig. 8).

Fits of two-phase regressions for each single site, for streams, and for the whole data set were all highly significant ($p < 0.001$; Table 5), indicating that in these two-phase, symmetrical trajectories, the survival rates of recruits were highest in the years of intermediate discharge (around 40.0 Hm^3) that occurred in the years 1987, 1988, 1996, and 2005 and were lowest at the two opposing extremes of the discharge in the driest springs of the years 1989 and 1997 and in the most rainy springs of 1995 and 2001.

Increasing and decreasing discharge conditions above the intermediate values appear to have symmetrical effects with comparable deviations above or below the average discharge resulting in comparable survival rates. However, the slopes of both ascending and descending portions differed significantly among sites. An ANCOVA for the ascending portion of the curve among the 12 sites indicated highly significant differences among the elevations ($F_{[11,122]} = 6.32$, $p < 0.001$) and among the slopes ($F_{[11,122]} = 7.91$, $p < 0.001$). Likewise, an ANCOVA for the descending portion indicated significant differences among the slopes ($F_{[11,45]} = 2.02$, $p = 0.03$). The strong effect of the site revealed not only that the lowest survival rates at minimum discharge differed among sites, but also, importantly, that the rates at which survival increased or decreased with the increasing or decreasing discharge differed markedly among sites.

Effects of discharge on year-class mortality and on the subsequent female spawner density

Given the importance of density of recruits for year-class mortality and for the subsequent spawner density and given the strong temporal synchrony revealed among the spatial pat-

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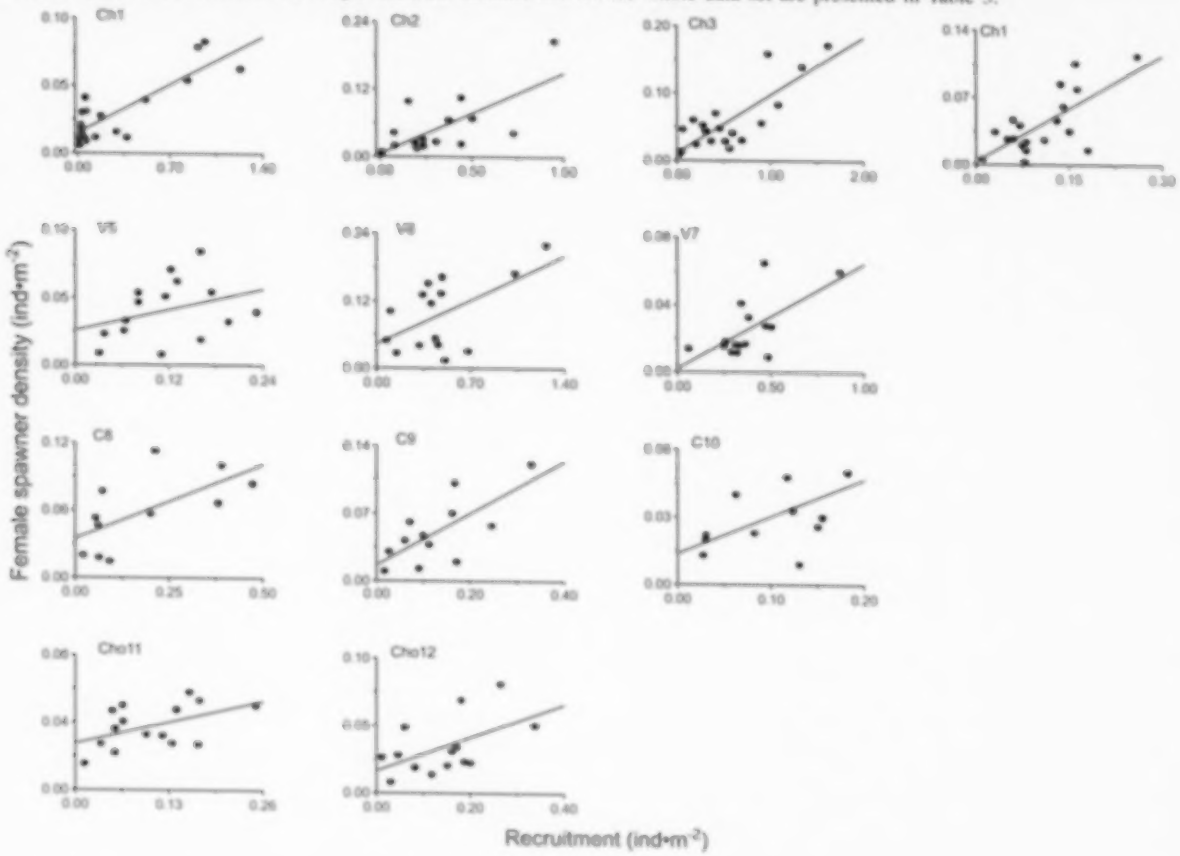
Fig. 6. Linear relationships between the subsequent spawner density (individuals (ind)·m^{-2}) and recruitment (ind·m^{-2}) across year classes of brown trout at 12 study sites of Rio Chaballos (Ch1–Ch4), La Viella (V5–V7), Castañedo (C8–C10), and Choudral (Cho11–Cho12). Regressions coefficients for single sites, for streams, and for the whole data set are presented in Table 3.



terms of mortality and spawner density, I explored whether the effects of discharge on the survival rates of the youngest juveniles were sufficiently strong to be reflected during their whole lifetime.

Both year-class mortality and the subsequent spawner density described similar two-phase relationships with discharge when trout emerge, and these patterns were strongly consistent for the three spatial scales examined (single sites, streams, and whole data set). The discharge–mortality and discharge – subsequent spawner density relationships for the whole data set are represented in Figs. 9 and 10.

Effects of habitat quality at the site scale on the spatial variations of recruitment, year-class mortality, and spawner density

The mean depth of the sites averaged over the whole study period as the simplest measure of the complex spatial structure of the sites was revealed as a strong predictor of the numerical variations across spatial scales. Mean recruitment was strongly related to site depth, as well as the slopes (positive or negative) of the discharge–recruitment relationships, mean year-class mortality, and the mean abundance of spawner females (Fig. 11).

The four site-specific population traits described two-phase trajectories over site depth, and fits of two-phase regressions revealed that 60.1% of the spatial variance in recruitment and 60.0% of the variance in the slopes of the discharge–recruitment curves were explained by the single effect of site depth. Moreover, 82.0% of the among-site variation in mortality and 57.8% of the variance in subsequent spawner density variations were also explained by the among-site variations in site depth (Table 6).

Discussion

This long-term study revealed notable variations in recruitment and density of stream-resident brown trout within the Rio Esva drainage across scales of space and time. Conventional hypothesis testing provided no evidence to support the hypothesis that year-to-year variation in annual recruitment was regulated by the operation of density dependence in the form of a stock–recruitment relationship. Likewise, tests for the operation of delayed density dependence provided no evidence to support the hypotheses that the abundance of female spawners surviving to spawn 2 years later is regulated by the original parental density. Nor was evidence

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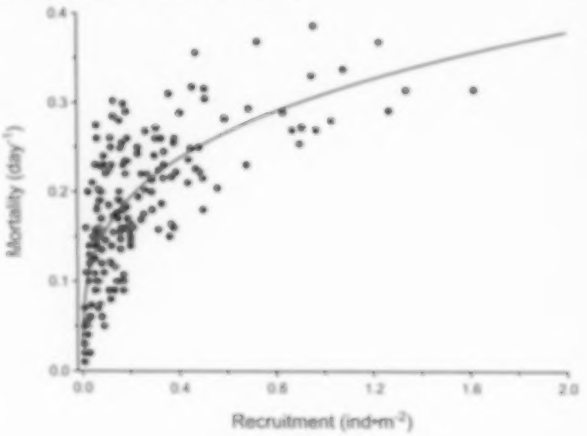
Table 3. Coefficients of the linear regressions between spawner density and recruitment of brown trout (see Fig. 6) for the 12 sites (1 to 12) of Rio Chaballos (Ch), La Viella (V), Castañedo (C), and Choudral (Cho).

	N	*A*	*B*	R^2	*p*
Rio Chaballos	66	0.021	0.069	49.7	<0.001
Ch1	17	0.015	0.046	66.7	<0.001
Ch2	15	0.015 NS	0.076	40.0	0.01
Ch3	17	0.009 NS	0.091	68.1	<0.001
Ch4	17	0.006 NS	0.365	69.5	<0.001
La Viella	44	0.022	0.100	27.9	<0.001
V5	14	0.019 NS	0.225	40.0	0.01
V6	14	0.040 NS	0.112	32.8	0.03
V7	14	0.002 NS	0.056	51.8	0.003
Castañedo	35	0.022	0.181	44.5	<0.001
C8	11	0.035	0.133	41.6	0.03
C9	12	0.016 NS	0.272	52.2	0.008
C10	12	0.014 NS	0.166	37.7	0.03
Choudral	28	0.023	0.108	24.0	0.008
Cho11	14	0.024	0.151	39.7	0.015
Cho12	14	0.016 NS	0.123	29.4	0.04
Total	171	0.026	0.077	33.2	<0.001

Note: *N*, the number of data pairs; *A* and *B*, the intercepts and slopes, respectively; R^2, the variance explained; *p*, the level of significance; NS, not significant.

Fig. 7. Power relationship between year-class mortality (Z, day^{-1}) during the second half of the lifetime and recruitment (R, individuals (ind)·m^{-2}) for 164 year classes of brown trout in Rio Chaballos, La Viella, Castañedo, and Choudral tributaries of Rio Esva (Asturias, northwestern Spain). The power regression is $Z = 0.311R^{0.291}$, $R^2 = 0.56$, $p < 0.001$.



provided for the occurrence of compensatory effects of recruitment dependence. The lack of compensatory density-dependent factors held over the increasingly larger spatial scales of this study ranging from the local scale of a single site to streams to the whole-river drainage.

In contrast, the analysis of environmental factors underlying population numbers across space and time matched and further extended the results of previous studies focused on one of the streams in this study (i.e., Rio Chaballos). Based on a 13-year data set at four study sites (Ch1 to Ch4 in this study), Lobón-Cerviá and Rincón (2004) noted that annual recruitment was determined by the area of spatial habitat suitable for the youngest juveniles, which is determined by the discharge conditions operating during or just after emergence. Further analysis of the self-thinning experienced by the same year classes and sites (Rincón and Lobón-Cerviá 2002) corroborated other studies (Lobón-Cerviá and Mortensen 2006) to offer evidence that at the scale of a stream site, the spatial habitat is size-limited and becomes increasingly limited for larger individuals, emphasizing the importance of spatial habitat availability for the larger (older) individuals.

The extended geographical scale of this study emphasized the role of single environmental factors as drivers of population size across substantially larger spatial and temporal scales. Rainfall, as a regional factor, operates on the baseflow conditions to determine annual-specific discharge conditions in March during the emergence period. Discharge determines the amount of spatial habitat suitable for the youngest juveniles and hence the strength of annual recruitment throughout the river drainage. Importantly, the effects of discharge on recruitment appeared sufficiently strong to be reflected on the mortality rates during the second half of their lifetime and on the subsequent density of female spawners. Thus, years of favorable rainfall–discharge conditions maximized annual recruitment to induce stronger year classes with more abundant female spawners. At the opposing ends of the discharge conditions, the most unfavorable years (too dry or too rainy during the emergence period) have similarly negative effects resulting in markedly lower annual recruitment and weaker year classes of lower spawner densities.

At the local scale of a stream reach, sites with similar ranges of parental densities, either within or among streams, showed persistently divergent annual recruitment magnitudes over temporal scales. Site-specific structural features related to mean depth apparently determined the carrying capacity of the sites through their effects on the availability of spatial habitat suitable for recruits. Within the range of sites in this study, sites with average depth provided a greater amount of suitable spatial habitat and hence greater recruitment, whereas shallower and deeper sites offered less abundant spatial habitat to sustain recruits. Site depth interacted with the year-to-year variations in discharge to augment or diminish the spatial habitat and hence the annual carrying capacity of the sites to sustain recruits. Favorable years augmented substantially the spatial habitat of sites with intermediate depth relative to shallower and deeper sites. As a consequence, favorable years result in marked differences in the carrying capacity of the sites and hence in recruitment, whereas such differences became obscured in unfavorable years when the carrying capacity declines at all sites and recruitment attains the lowest magnitudes.

Thus, recruitment essentially reflects the environmental patchiness that typifies stream gradients (Pringle et al. 1988) that in turn reflects stream-specific characteristics. The relative importance of site depth and the similar effects of shallower and deeper sites on recruitment means that naturally shallow streams (i.e., Choudral) tend to have shallower sites and hence a lower carrying capacity and lower recruitment comparable in magnitudes to the opposite condition, deeper

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Table 4. Coefficients of determination (R^2, %) obtained from pairwise correlations (all positive) among the indices of (*a*) survival rates of recruits (*R*), (*b*) survival rates of the subsequent female spawner density (SA), and (*c*) the mortality rates (*Z*) among the 12 study sites (1 to 12) of Rio Chaballos, La Viella, Castañedo, and Choudral across year classes (in parentheses).

(*a*) Survival rates of recruits.

	*R*2 (18)	*R*3 (20)	*R*4 (20)	*R*5 (17)	*R*6 (17)	*R*7 (17)	*R*8 (13)	*R*9 (14)	*R*10 (14)	*R*11 (17)	*R*12 (16)
*R*1(20)	**43**	**22**	**19**	**26**	**26**	**31**	**42**	8	15	**57**	**35**
*R*2(18)		**41**	**49**	**25**	**71**	**73**	**39**	10	**53**	**45**	**60**
*R*3(20)			**32**	**38**	**43**	**72**	**54**	**29**	**34**	**22**	**55**
*R*4(20)				**49**	**43**	**62**	**50**	20	**31**	**37**	**38**
*R*5(17)					21	**41**	**40**	**54**	**31**	6	13
*R*6(17)						**68**	**38**	10	**49**	**50**	**70**
*R*7(17)							**50**	21	**36**	**39**	**53**
*R*8(13)								**35**	**36**	**37**	**60**
*R*9(14)									**55**	2	5
*R*10(14)										10	34
*R*11(17)											**58**

(*b*) Survival rates of the subsequent female spawners.

	SA2 (15)	SA3 (17)	SA4 (17)	SA5 (14)	SA6 (14)	SA7 (14)	SA8 (11)	SA9 (12)	SA10 (12)	SA11 (14)	SA12 (14)
SA1(17)	**56**	**40**	**47**	12	8	**25**	11	**30**	2	13	2
SA2(15)		**57**	**53**	6	**64**	**52**	**49**	**45**	**38**	13	8
SA3(17)			16	15	**45**	**38**	**54**	20	5	17	**46**
SA4(17)				22	**56**	**38**	26	**52**	**33**	26	14
SA5(14)					**49**	**32**	**51**	**43**	29	14	9
SA6(14)						**38**	**60**	**65**	**53**	12	**30**
SA7(14)							**37**	10	13	**63**	**47**
SA8(11)								**50**	26	**38**	**61**
SA9(12)									**61**	13	15
SA10(12)										**62**	29
SA11(14)											**65**

(*c*) Mortality rates.

	*Z*2 (15)	*Z*3 (17)	*Z*4 (17)	*Z*5 (13)	*Z*6 (13)	*Z*7 (13)	*Z*8 (11)	*Z*9 (11)	*Z*10 (11)	*Z*11 (13)	*Z*12 (13)
*Z*1(17)	**55**	**35**	**28**	**76**	**31**	**40**	9	20	**43**	**35**	31
*Z*2(15)		**41**	**31**	**43**	12	**56**	**43**	**42**	**52**	**34**	29
*Z*3(17)			**30**	12	28	8	**45**	7	19	**36**	5
*Z*4(17)				26	**41**	28	22	18	16	**35**	3
*Z*5(13)					**47**	**47**	8	30	**38**	**51**	24
*Z*6(13)						19	23	29	**46**	**59**	**32**
*Z*7(13)							**36**	**51**	**38**	**42**	**33**
*Z*8(11)								26	**44**	26	23
*Z*9(11)									**60**	**52**	**37**
*Z*10(11)										**56**	**59**
*Z*11(13)											**50**

Note: Levels of significance are indicated as $0.001 < p < 0.05$ in bold, $0.05 < p < 0.10$ underlined, and $p > 0.10$ all others.

streams with deeper sites (i.e., Castañedo). And streams of intermediate depths and sites with intermediate depths maximize the carrying capacity and, hence, recruitment.

Highly significant correlations among the indices of survival rates of recruits and of the subsequent spawner density and also among the mortality rates underscored strong spatial synchrony in the temporal dynamics of the year classes (Lobón-Cerviá 2004). Such synchrony is first accounted for by environmental factors and then by the operation of density dependence. The timing of hatching and emergence in brown trout is determined by stream temperature (see Elliott (1981) for a review). In Rio Esva, emergence, influenced by a common temperature regime, occurred simultaneously throughout the river drainage (Lobón-Cerviá and Rincón 1998; Lobón-Cerviá 2000). During the emergence period, the spatial habitat suitable for the youngest juveniles imposed by discharge synchronized recruitment throughout the river drainage. After a prolonged period of no mortality but intensive growth (Lobón-Cerviá 2003, 2005), during which time available resources did not affect survival but did af-

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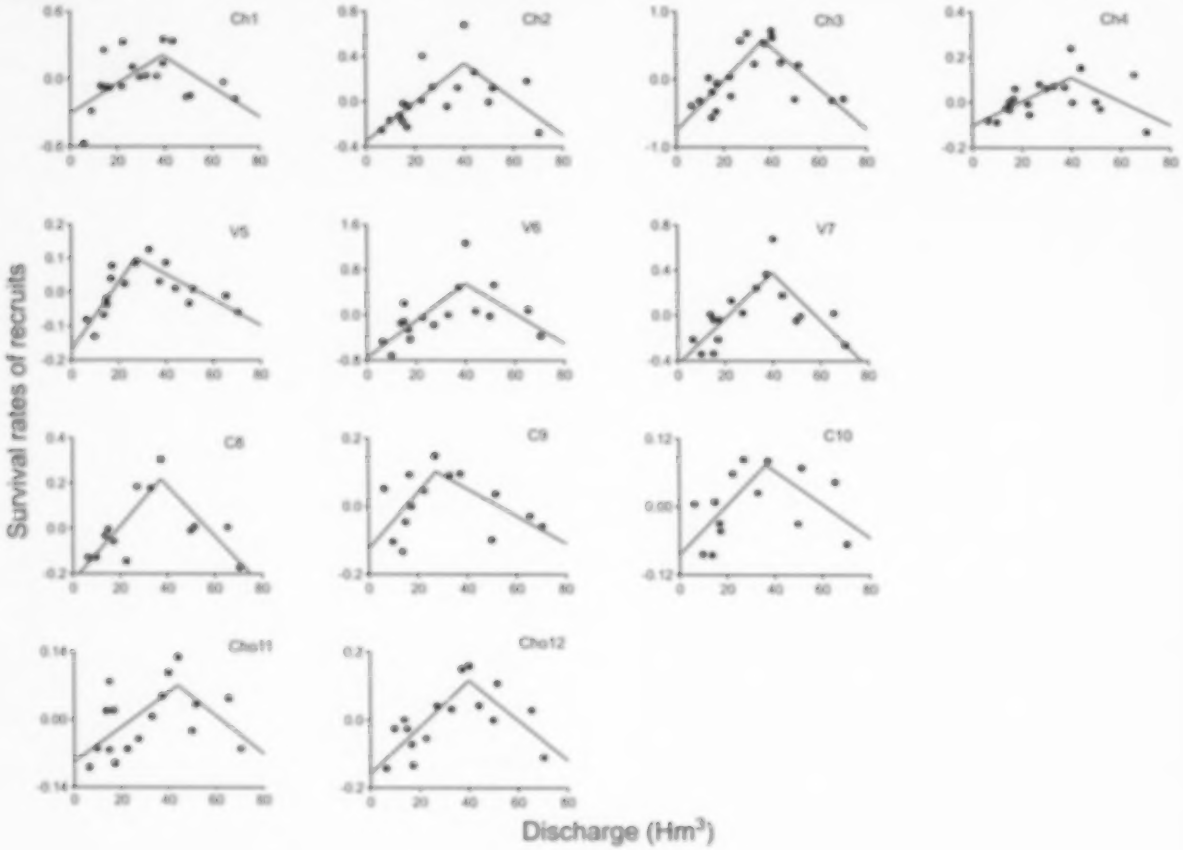
Fig. 8. Two-phase trajectories depicted by the indices of survival rates of recruits of brown trout (individuals·m^{-2}) over discharge (Hm^3) in March at 12 study sites of Río Chaballos (Ch1–Ch4), La Viella (V5–V7), Castañedo (C8–C10), and Choudral (Cho11–Cho12) tributaries of the Río Esva drainage (Asturias, northwestern Spain). Regression coefficients, variance explained, and significance levels are presented in Table 5.



fect growth (Lobón-Cerviá 2005), the year classes reached the carrying capacity of the sites when individuals attained a given size (Rincón and Lobón-Cerviá 2002; Lobón-Cerviá and Mortensen 2006). The onset of mortality begins when these larger individuals encounter the size-limited spatial habitat, which is increasingly limited for larger individuals (J. Lobón-Cerviá, unpublished data). Once these threshold sizes were reached, high mortality persisted until the total disappearance of the year class.

During the second half of the lifetime, density-dependent mortality adjusted the number of individuals that could be accommodated in the spatially limited habitat and all individuals in excess were doomed to die. Thus, density dependence appears as an epiphenomenon that eliminates the excess individuals and its intensity, weak at sites and years of low recruitment and stronger at sites and years of higher recruitment, eventually synchronizes the numerical dynamics across sites. Importantly, such density-dependent mortality provides no feedback among year classes given that the new recruitment derived from the surviving female spawners will be determined by the future discharge conditions.

Although no previous study has examined environmental drivers of population size at the spatial and temporal scales of this study, the outstanding role of discharge for the survival of the youngest juveniles has been reported for native populations (Solomon and Patterson 1980; Jensen and Johnsen 1999; Cattanéo et al. 2002) and for populations introduced across geographical regions (Nuhfer et al. 1994; Spina 2001; Carline 2006). Habitat quality related determinants of spatial variations in density have been documented within the native distributional range (Milner et al. 1993) and other regions (Beard and Carline 1991; Hayes et al. 1996). The importance of site depth as a surrogate of living space has been emphasized in the literature for brown trout (Chapman 1966; Bohlin et al. 2001) and other salmonids (Hunt 1971; Rinne and Medina 1988) and, in turn, has been frequently obscured by studies that failed to detect its effects relative to other habitat variables (see Fausch et al. (1988) for a review). Such discrepancies may have been caused by temporal variations of recruitment and density. In favorable years, the among-site difference in recruitment becomes overwhelming, whereas intermediate years or years located at the two extremes of discharge conditions tend to homogenize the levels of recruitment by offering lower and similar magnitudes across sites that necessarily obscure the effects of site depth. Thus, short-term studies in years when recruit-

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Table 5. Coefficients of two-phase regressions fitted to the survival rates of brown trout recruitment versus discharge in March for each site separately (see Fig. 8), for single streams, and for the whole data set.

	N	A_1	B_1	K	B_2	R^2
Rio Chaballos	78	–0.355	0.017	39.7	–0.017	42.1
Ch1	20	–0.305	0.013	39.1	–0.013	37.8
Ch2	18	–0.352	0.017	40.0	–0.016	51.7
Ch3	20	–0.757	0.037	36.6	–0.030	67.1
Ch4	20	–0.103	0.005	40.0	–0.005	41.5
La Viella	51	–0.420	0.019	40.0	–0.017	42.5
V5	17	–0.136	0.008	28.1	–0.004	67.4
V6	17	–0.752	0.033	40.0	–0.026	55.5
V7	17	–0.431	0.020	39.8	–0.021	69.1
Castañedo	41	–0.137	0.008	35.3	–0.006	43.9
C8	13	–0.231	0.012	37.3	–0.011	68.9
C9	14	–0.125	0.008	27.4	–0.004	39.0
C10	14	–0.085	0.004	36.5	–0.003	40.9
Choudral	34	–0.125	0.005	40.0	–0.004	50.7
Cho11	17	–0.087	0.004	44.0	–0.004	39.2
Cho12	17	–0.159	0.007	40.0	–0.006	64.5
Total	204	–0.285	0.013	40.0	–0.013	36.0

Note: All fits are significant at $p < 0.001$. N, the number of year classes; A_1, elevation; B_1 and B_2, the two slopes of two-phase model (i.e., eq. 2); K, the breakpoint upon which the regression changes direction; R^2, the variance explained.

Fig. 9. Two-phase relationship between mortality rates (day^{-1}) during the second half of the lifetime of 164 year classes of brown trout and discharge (Hm^3) in March. Regression coefficients are $A = 0.098$, $B_1 = 0.004$, $B_2 = -0.004$, and the breakpoint $K = 40.3$; significant at $p < 0.001$.

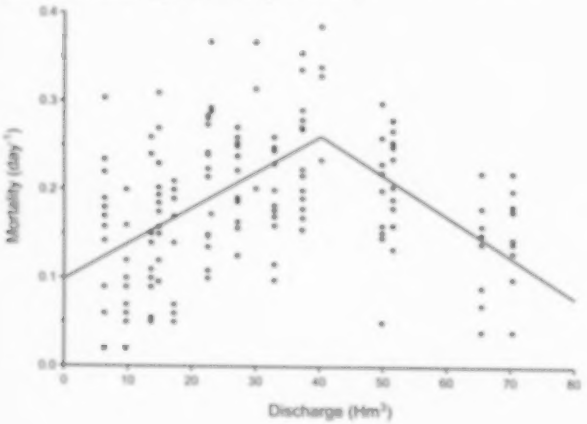
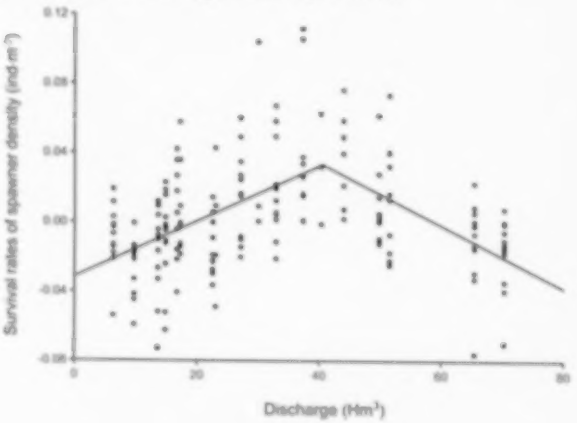


Fig. 10. Two-phase relationship between the subsequent spawner density (individuals (ind)·m^{-2}) of 171 year classes of brown trout and discharge (Hm^3) in March for the whole data set. Regression coefficients are $A = -0.043$, $B_1 = 0.002$, $B_2 = -0.002$, and the breakpoint $K = 41.7$; significant at $p < 0.001$.



ment is not maximized may obscure the effects of site depth but identify the effects of other habitat variables.

Environmental determinants of population size imply an acute instability over time to an extent that these populations might be driven to extinction if unfavorable rainfall–discharge conditions prevail over prolonged time periods. However, the long-term persistence of these populations, known in these rivers since historical times, suggests the occurrence of response mechanisms. During the study, the greatest recruitment observed at a site scale was 224 individuals at site V6 and only 80 individuals at site Ch4. In salmonids, reproductive events in which eggs show markedly high survival rates is a well-documented pattern (Ricker 1954; Le Cren 1965; Crisp 1993). Consistent with this pattern, in Rio Esva, no more than just a few females at the site scale successfully spawning 50–250 eggs·female^{-1} (Lobón-Cerviá et al. 1997) appears sufficient to attain the recruitment levels observed across years and sites. Linear relationships between recruitment and the subsequent spawner densities with low mortalities at low recruitment levels might guarantee a minimum number of spawning females, with further enhanced survival by the few females that spawn twice at given sites and years. This apparently provides a mechanism to respond to prolonged unfavorable environmental conditions, reducing the risk of extinction.

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Fig. 11. Two-phase relationships between mean depth (cm) of the study sites and the four major brown trout population traits: (*a*) mean recruitment (individuals (ind)·m^{-2}) averaged over the study years, (*b*) slopes (positive or negative in Fig. 8) of the discharge–recruitment relationships, (*c*) mean year-class mortality rates (day^{-1}), and (*d*) the mean subsequent spawner density (ind·m^{-2}). Coefficients of the two-phase regressions are presented in Table 6.

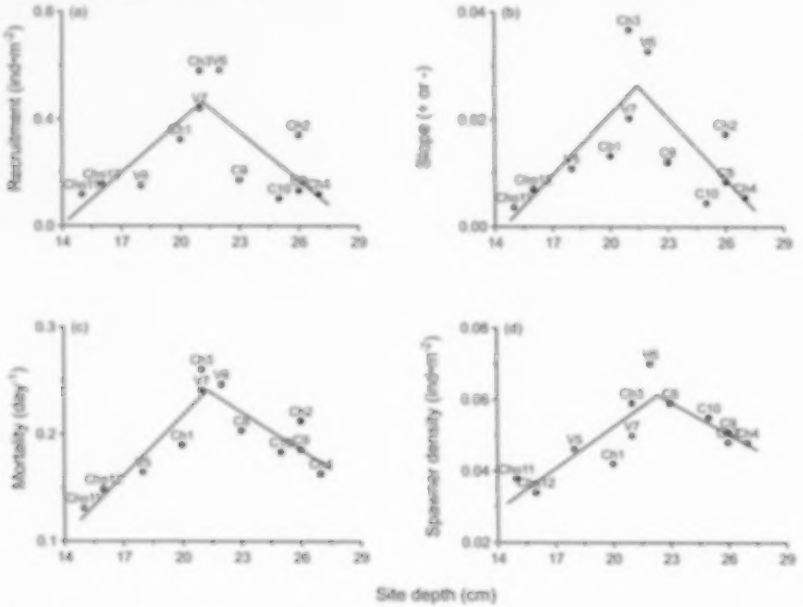

Table 6. Coefficients of the two-phase regressions fitted to the mean annual recruitment of brown trout, slopes of discharge–recruitment relationships (positive or negative), mean subsequent spawner density and mean year-class mortality rates versus site depth (see Fig. 11).

	N	A_1	B_1	K	B_2	R^2
Recruitment	12	–0.901	0.065	21.1	–0.061	60.1
Slopes (+ or –)	12	–0.055	0.004	21.4	–0.004	60.0
Mortality rates	12	–0.157	0.019	21.3	–0.012	82.0
Spawner density	12	–0.025	0.004	22.3	–0.003	72.8

Note: N, the number data pairs (sites); A_1, elevation; B_1 and B_2, the positive and negative slopes, respectively; K, the breakpoint upon which the slope changes direction; R^2, variance explained. All fits are significant at $p < 0.001$.

In a broader perspective, this study also provides insight into the general elucidation of the rules underlying animal population size, persistence, and resilience and to delineate further studies. Recent attempts to reconcile the roles of density-dependent and density-independent processes have suggested that population dynamics result from the interaction between deterministic (density-dependent) nonlinear skeletons and (density-independent) noise (Coulson et al. 2004; Boyce et al. 2006) in which the two suites of factors may have equal importance (Bjornstad and Grenfell 2001). Consistent with this view, the "Moran theorem" (Moran 1953; Lundberg et al. 2000) predicts that synchronization of disjunct populations may occur if the local populations share the same density-dependent structure and the same environmental variability (Lundberg et al. 2000). That is, populations subject to the same density-dependent structure (i.e., skeleton) may show spatial synchrony because stochastic but synchronous climatic effects operate simultaneously across populations (Liebhold et al. 2004; Hugueny 2006), a phenomenon that has been documented for a variety of taxa (Ranta et al. 1998; Liebhold et al. 2004), including brown trout populations across a broad spatial scale (Cattanéo et al. 2003).

The present study showed that the number of brown trout individuals in a given space and time resulted from the combined effects of density-independent and density-dependent factors. However, the two suites of factors operated in a manner just opposite to that predicted by the theoretical studies above. In the Rio Esva brown trout, the structure (i.e., skeleton) is built "a priori" by the stream and site characteristics and varies over time, tracking regional factors such as discharge through their effects on the availability of spatial habitat upon which density dependence operates "a posteriori" to adjust the numbers that exceed the maximum numbers established by the availability of spatial habitat. Consequently, the operation of single environmental factors such as discharge and site depth followed by the operation of density dependence not only explains population numbers

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across spatial and temporal scales, but also explains temporal synchrony across spatial scales.

Marine and freshwater fish populations have been extensively studied (Cushing 1996; Matthews 1998). Although evidence for the importance of climatic factors for the population dynamics of marine populations is steadily accumulating (Glantz 1992; Cushing 1996), comparable environmental determinants of population size in streams and other freshwater systems remain elusive. This may not be surprising given the lack of studies over sufficiently long temporal scales to capture ranges of favorable and unfavorable years, a prerequisite to detecting changes in population size. Remarkably, population studies in which sufficient environmental variability has not been captured may confound the effects of stochastic processes with patterns typical of density-dependent population regulation. At the study sites of Rio Esva, brown trout densities varied over time within moderate limits and returned rapidly to a sort of "average density" after strong departures caused by sharp changes of rainfall–discharge conditions. This resulted from the natural trend of rainfall–discharge conditions to be similar year after year (an obvious meteorological phenomenon across climatic regions), whereas the opposing extremes of either too dry or too rainy springs occurred at irregular intervals and lasted for very short time periods. Limited temporal variations in environmental conditions and a rapid recovery may obscure the expected "statistically random walk" that typifies nonequilibrium populations, causing then to resemble bounded variances typical of regulated populations. Likewise, if a short-term study accidentally captures a few years of extreme climatic conditions and, hence, extremes of annual recruitment, as for example those recorded in Rio Esva in 1989 vs. 1990 and a decade later in 2003 vs. 2004 and 2005, and the study continues over the next few years of intermediate environmental conditions, then the variance in density will not necessary increase over the next few years but may even decrease with time, clashing with a major attribute of nonequilibrium populations (Murdoch 1994).

Clearly, long-term studies are needed to elucidate the role of environmental factors underlying population size, and the longer the time period, the greater the chance of capturing environmental and population variability and the greater the chance of detecting environmental determinants of population size.

Acknowledgments

This study was funded by three CICYT projects (Ref. Nos. PB84-0142, P1389-0048, and PB92-0045). Administrative permissions for fishing were obtained by agreement between CSIC and Principado de Asturias. Key factors for the long-term success of this study include the ever-helpful assistance of the Municipality of Valdés and the continuous support of numerous individuals. Regrettably, the list is too long to be included here but they all know how I appreciate their efforts. Comments by Dr. Robert J. Wootton and two anonymous reviewers improved substantially an earlier draft. Ms. Maite Lavandeira and the American Journal Experts Co. did their best to improve my English.

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Historical analysis of *Pan* I in Atlantic cod (*Gadus morhua*): temporal stability of allele frequencies in the southeastern part of the species distribution

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Abstract: We investigated temporal genetic differentiation at the pantophysin (*Pan* I) locus in four Atlantic cod (*Gadus morhua*) populations from the southeastern part of the species distribution: the Baltic Sea, the North Sea, the Faroe Plateau, and the Faroe Bank. Historical otolith collections enabled investigation of allele frequency variation over time periods up to 69 years employing *Pan* I primers specifically designed for partially degraded DNA. Small and nonsignificant temporal changes in *Pan* I allele frequencies were observed in the four populations. Simultaneous microsatellite analysis revealed similar temporal genetic stability with temporal F_{ST} values ranging from 0 to 0.006, suggesting limited demographic changes. Sea surface temperature, which has been suggested as the primary driver for the geographical distribution of *Pan* I alleles in cod, showed no long-term trend although temperature has increased since the mid-1990s. Our study demonstrates that populations in the southeastern part of the species range has been characterized by very high frequencies of the *Pan* I^A allele for many decades, and accordingly, *Pan* I serves as a reliable marker for genetic stock identification on a macrogeographical scale.

Résumé : Nous étudions la différenciation génétique au locus *Pan* I chez quatre populations de morues franche (*Gadus morhua*) du sud-est de l'aire de répartition de l'espèce, soit celles de la Baltique, de la Mer du Nord, du plateau de Féroé et du banc de Féroé. Des collections historiques d'otolithes ont permis d'analyser la variation de la fréquence des allèles sur des périodes pouvant atteindre 69 ans à l'aide d'amorces *Pan* I spécialement conçues pour l'ADN partiellement dégradé. Nous observons des changements temporels faibles et non significatifs dans la fréquence des allèles de *Pan* I dans les quatre populations. Des analyses simultanées des microsatellites montrent aussi une stabilité génétique temporelle, les valeurs temporelles de F_{ST} variant de 0–0,006, ce qui indique de faibles changements démographiques. La température de surface de la mer, qu'on a suggéré comme principal facteur explicatif de la répartition géographique des allèles de *Pan* I chez la morue, ne montre aucune tendance à long terme, bien que la température ait augmenté depuis le milieu des années 1990. Notre étude démontre que les populations du sud-est de l'aire de répartition de l'espèce sont caractérisées depuis plusieurs décennies par de hautes fréquences de l'allèle *Pan* I^A; c'est pourquoi *Pan* I peut servir de marqueur fiable de l'identification génétique des stocks à l'échelle macrogéographique.

[Traduit par la Rédaction]

Introduction

Noncoding DNA markers such as microsatellites have become the preferred option for inferring population structure in marine organisms. In particular for marine fishes, the application of microsatellites for population genetic studies has generated many new insights (see reviews by Waples 1998; DeWoody and Avise 2000). The discovery of genetic structure on most geographical levels investigated in marine fishes (e.g., Nielsen et al. 2003; O'Reilly et al. 2004; Jørgensen et al. 2005) has opened a wealth of new opportunities for genetic stock identification (GSI) in marine fishes. Among the most promising possibilities are the assignment of individuals to population (reviewed by Hansen et al. 2001; Manel et al. 2005) and estimation of the proportion contributed by potential donor populations to a mixed fishery, so-called mixed stock analysis (e.g., see Pella and Masuda 2001). The power of these statistical methods depends critically on the level of genetic divergence among populations. If microsatellite differentiation is low, as commonly found among populations of marine fishes, the number of loci has to be increased to improve exclusion power

Received 7 November 2006. Accepted 24 May 2007. Published on the NRC Research Press Web site at cjfas.nrc.ca on 12 October 2007. J19649

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 doi:10.1139/F07-104 © 2007 NRC Canada

(Manel et al. 2005). Unfortunately, also the time and cost of conducting the genetic analysis increases, making them less attractive for real time and routine application.

Alternatively, it is possible to turn to genes under directional selection and (or) genetic markers associated with genes under selection, which are expected to show higher population divergence than neutral markers. In particular for organisms characterized by high effective population sizes, such as marine fishes, selection is a much more powerful force than random genetic drift for driving population divergence (see Endler 1986).

Recent studies have shown that it is possible to identify microsatellite loci under selection in marine fishes (Nielsen et al. 2006); however, the selected genetic marker that has attracted most attention recently for marine fishes is the pantophysin (*Pan* I) locus in gadoid fishes. Pogson et al. (1995) sequenced the anonymous cDNA clone GM798, which showed elevated levels of genetic differentiation compared with a number of other cDNA clones and identified the gene as synaptophysin (*Syp* I) — later revised to *Pan* I (Pogson 2001). *Pan* I is an integral membrane protein of unknown function (see Pogson and Mesa 2004 and references therein for further details about protein structure and suspected function). By investigating inter- and intra-specific sequence divergences, positive Darwinian selection has been invoked (Pogson 2001; Canino and Bentzen 2004; Pogson and Mesa 2004). In cod, two highly different allele classes have been identified, the *Pan* I^A and the *Pan* I^B alleles respectively, which differ by four amino acid substitutions and can be differentiated by a *Dra* I restriction site. The two alleles appear to have evolved prior to the split between cod and its sister taxon walleye pollock (*Theragra chalcogramma*) (Pogson and Mesa 2004). Within the two classes, additional sequence variation can be found (see Pogson 2001), but the major focus of population genetic studies has been on the distribution of the diallelic system only, revealing extensive *Pan* I differentiation in cod (for an overview see Case at al. 2005). Whether this distributional pattern is caused by contemporary or historical patterns of selection is difficult to demonstrate (Pogson 2001), but contemporary selection at *Pan* I has been invoked as responsible for growth variation among populations (Fevolden and Pogson 1995) and individuals (Case et al. 2006), as well as inducing temporal instability in allele frequencies (Karlsson and Mork 2003).

Potential environmental drivers responsible for selection at *Pan* I were investigated in a recent paper by Case et al. (2005). They found *Pan* I allele frequencies to be correlated with temperature, salinity, and depth, of which the latter was also suggested recently by Pampoulie et al. (2006). Case et al. (2005) concluded that "In areas of 'normal' salinity, temperature may be the determining factor in *Pan* I allele frequencies." Short-term (interannual) fluctuations in ocean temperatures are well documented as well as longer term trends (Stenseth et al. 2004; B.R. MacKenzie and D. Schiedek, unpublished data), which may be due to global warming (Barnett et al. 2005). Therefore, the expectations are temporally changing patterns of selection, which could lead to fluctuating or gradually changing allele frequencies, as suggested by Karlsson and Mork (2003). Temporal changes in allele frequencies may have an important bearing for invoking population of origin of individuals or groups of individuals, thereby leading to biased or erroneous GSI estimates. Accordingly, to employ genetic markers under selection for GSI, it is important to identify long-term patterns of stability to determine the effects of the environmental driver(s) of selection, but also to rule out demographic factors (i.e., migration and genetic drift as sources of temporal variation).

DNA analyses from historical collections (scales and otoliths; e.g., see Nielsen et al. 1997; Hutchinson et al. 1999) of fish have shown much promise for investigating patterns of temporal genetic variation. In marine fishes, archived material has been used to evaluate long-term (putative) neutral variation by estimation of genetically effective population sizes and migration (Hauser et al. 2002; Hutchinson et al. 2003; Poulsen et al. 2006). Until now, however, no attempts have been made to evaluate historical patterns of selection on specific candidate loci in marine fishes.

Here we employ historical cod otolith samples spanning up to 69 years to evaluate long-term temporal stability in *Pan* I allele frequencies in populations from the southeastern part of the species' distributional range. We use new primers specifically designed for analysis of partially degraded DNA from otoliths. We investigate the relative roles of neutral (drift and migration) and selective evolutionary forces by simultaneously analyzing noncoding microsatellites. We also explore the potential role of thermal selection in shaping patterns of *Pan* I allele frequencies by correlating historical temperature regimes with temporal development of *Pan* I allele frequencies. Finally, based on the current results, we evaluate the suitability of *Pan* I and other selected markers for GSI in marine fishes.

Materials and methods

Sample collection

A total of 442 cod from four populations was analyzed for *Pan* I variation: the Baltic proper (Bornhom Basin), the North Sea (Moray Firth), the Faroe Plateau, and the Faroe Bank (see Fig. 1 and Table 1). The four areas are important spawning grounds for cod, but have all suffered from overexploitation in recent years. Contemporary samples were collected from primarily adult mature individuals and consisted of gill tissue stored in ethanol. Samples from the Baltic Sea and the North Sea were collected in relation to previous research projects (for details about samples, see Nielsen et al. 2001, 2003; Poulsen et al. 2006), while the contemporary samples from Faroese waters were collected by the University of the Faroe Islands in cooperation with the Faroese Fisheries Laboratory. Historical samples consisted of saggital otoliths stored in paper bags and provided by the Danish Institute for Fisheries Research, Fisheries Research Services Scotland, and the Faroese Fisheries Laboratory.

Sea surface (0–2 m) temperature (SST) data were obtained from the International Council for the Exploration of the Sea (ICES) Hydrographic Data Centre and the Faroese Fisheries Laboratory for three regions corresponding to spawning areas for the sampled cod populations. The three areas were the

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Fig. 1. Location of temporal Atlantic cod (*Gadus morhua*) samples and *Pan* I allele frequencies. Grey: *Pan* I^A; black: *Pan* I^B. For list of sample abbreviations, see Table 1.

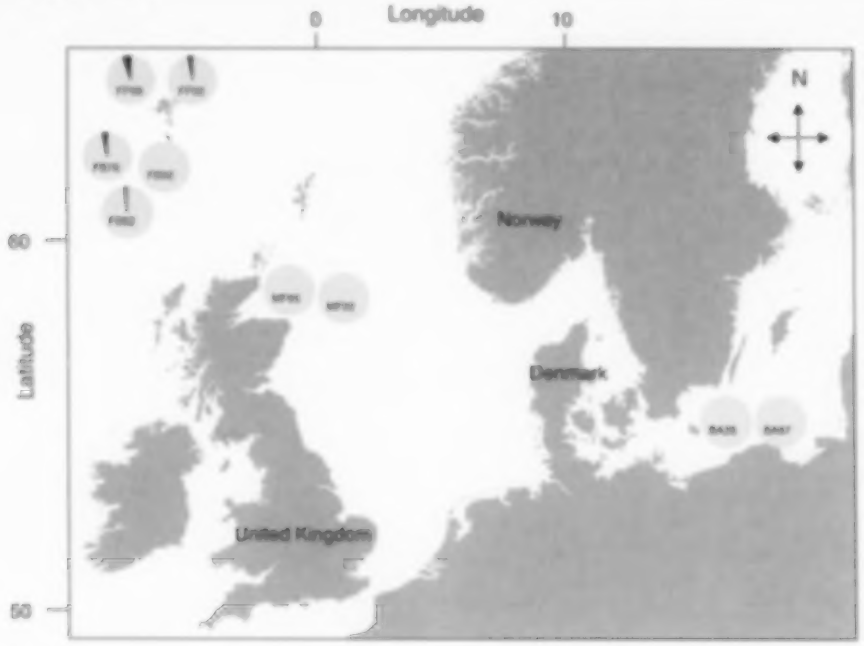

Table 1. Summary statistics of contemporary (and historical in parentheses) Atlantic cod (*Gadus morhua*) samples analyzed for *Pan* I (microsatellites) and frequencies of the *Pan* I^A allele.

Geographical locality	Position	Sampling year	Abbreviation	Month of sampling	No. of individuals	*Pan* I^A
Baltic Sea	Bornholm Basin	1928	BA28	NA	50 (50)	1
	(54.51°N, 15.28°E)	1997	BA97	April	50 (62)	1
North Sea	Moray Firth	1965	MF65	May	49 (50)	1
	(57.95°N, 2.20°W)	2002	MF02	March	50 (71)	1
Faroe Plateau	Faeroe Plateau	1969	FP69	April	50 (33)	0.95
	(62.52°N, 6.18°W)	2002	FP02	April	48 (45)	0.97
Faroe Bank	Faroe Bank	1978	FB78	April	56 (46)	0.97
	(60.94°N, 8.57°W)	1992	FB92	April	49 (0)	1
		2002	FB02	April	50 (71)	0.99

Bornholm Basin (55.9°N–54.9°N, 16.3°E–14.7°E), northwest North Sea (5°W–0°; 55°N–60°N), and the Faroe Plateau. The Faroese data are based on daily measurements at Mykines (7.672°W, 62.097°N) during 1914–1969 and at Oyragjógv (7.170°W, 62.116°N) 1990–2005. Data for the intervening years are derived from a validated oceanographic model of the local circulation (Hatun et al. 2005). Temperatures on the Faroe Bank are 1–2 °C warmer than those on the Plateau (Westerberg 1990; Lastein 1992) and are closely correlated with Plateau temperatures (E. Magnussen, unpublished data). The month chosen for comparison with genetic data was June, which approximates the timing of peak abundance of early life history stages (eggs and newly hatched larvae) for most of these regions (but not Bornhom Basin). It can be debated if June temperature per se could be the selective agent responsible for geographic differences in *Pan* I allele frequencies. However, reporting of June temperatures enabled direct comparison of our results with the most extensive study of relationships between temperatures and *Pan* I allele frequencies reported by Case et al. (2005).

DNA analysis

DNA was extracted from both contemporary and historical samples using a proteinase K – chelex procedure following Estoup et al. (1996). Owing to the degraded nature of DNA from archival material (Nielsen et al. 1999), we were unable to use previously designed primers for amplification of the *Pan* I locus. Based on one of the sequences from Pogson and Fevolden (2003) deposited in GenBank (Accession number AY146692), we designed primers for a 142-basepair (bp) segment spanning the diagnostic *Dra* I restriction site discriminating between the *Pan* I^A and the *Pan* I^B alleles (forward: 5′-GGCAAATGAAACCCAGAAAA; reverse: 5′- ATGACACTTGTGGCAAGCAG).

Polymerase chain reaction (PCR) amplification of the *Pan* I segment was conducted using standard reagents and an an-

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nealing temperature of 55° with 30 and 40 PCR cycles for contemporary and historical samples, respectively. Restriction analysis was carried out according to manufacturer's recommendations (Sigma), and fragments were visualized on a 2% agarose gel (NuSieve, Cambrex Corp.) stained with ethidium bromide.

PCR amplification of nine highly polymorphic microsatellites were conducted for the Baltic Sea and North Sea samples: *Gmo*2 (Brooker et al. 1994); *Gmo*19, *Gmo*34, *Gmo*35 (Miller et al. 2000); *Tch*5, *Tch*11, *Tch*14, *Tch*22 (O'Reilly et al. 2000); and GADM1 (Hutchinson et al. 2001). These results have previously been used to infer effective population sizes in cod populations (Poulsen et al. 2006). We also employed nine loci for the Faroe Bank samples; however, *Tch*22 was exchanged with *Gmo*8 (Miller et al. 2000). We did not perform microsatellite analysis on the intermediate (1992) samples, since our results from 1978 and 2002 revealed lack of temporal differentiation. The historical Faroe Plateau samples only allowed consistent amplification of three microsatellite loci with short maximum allele sizes because of bad DNA quality. These were *Gmo*2, with a maximum observed allele size of 135 bp; *Gmo*34 (maximum 119 bp); and *Gmo*35 (maximum 142 bp). Accordingly, the temporal tests for genetic differentiation at the Faroe Plateau are only conducted on three loci (see Table 2).

For both *Pun* I and microsatellite analysis, all runs included negative and positive controls, the latter consisting of individuals with known genotypes. Approximately 25% of the historical samples and 15% of contemporary samples were rerun to check for consistency of results. No signs of contamination were observed.

Statistical analysis

Tests for deviations from Hardy–Weinberg equilibrium for *Pun* I and individual microsatellite loci in each sample were conducted using the program FSTAT (Goudet 1995). The same program was also used to provide unbiased estimates of temporal, pairwise F_{ST} values for microsatellites, following Weir and Cockerham (1984), and associated 95% confidence intervals by bootstrapping over loci. Accordingly, no confidence interval was calculated for the Faroe Plateau data (i.e., only three loci; see Table 2). Finally, temporal heterogeneity in allele frequencies was tested by permuting alleles among samples (10 000 times).

The raw SST data were averaged to monthly values and plotted as time series to enable visual inspection for overall trends and variations at multiannual time scales. We also used statistical methods to investigate whether significant ($\alpha = 0.05$) variations occurred at multiannual time scales. These methods included linear regression for the detection of trends over the entire time period and general additive models (GAMs; Swartzman et al. 1992; Begg and Marteinsdottir 2002) for detection of variations at shorter (multiannual) time scales. The methodology used for fitting the GAMs to the SST data is described elsewhere (B.R. MacKenzie and D. Schiedek, unpublished data); in brief, the GAMs were fitted using a generalized cross-validation procedure applied to a locally weighted least squares regression. The variance (deviance) explained by these models was tested for statistical significance using the pseudo-R^2 criterion (Swartzman et al. 1992; B.R. MacKenzie and D. Schiedek, unpublished data).

Table 2. Temporal estimates of microsatellite intrapopulation differentiation (θ_{ST}), 95% confidence intervals (CI), and associated p values for pairwise exact tests of differentiation.

Temporal comparison	Baltic Sea	North Sea	Faroe Plateau	Faroe Bank
θ_{ST}	0.006	0.001	0.000	0.006
95% CI	0–0.012	0–0.06	—	0–0.023
p	<0.01	0.02	0.41	0.15

Results

As expected from the sequence information, the restriction analysis of the PCR-amplified *Pun* I segment provided a single band of 142 bp for *Pun* I^A homozygotes, two bands of 40 and 102 bp for *Pun* I^B homozygotes, and all three bands for heterozygotes. Generally, high amplification success was achieved from close to 100% for contemporary samples down to 96% for the FP69 sample. No significant deviations from Hardy–Weinberg proportions were found for *Pun* I; however, a single *Pun* I^B homozygote was found in two historic samples (FP69 and FB78). The amplification success of microsatellites was generally good, with less than 5% missing single locus genotypes. Only the FP69 sample had a high frequency of missing genotypes. For this sample, one-third of the individuals did not provide any amplification product for two or all three loci after two rounds of attempted amplification, and all results from these individuals were discarded accordingly. For the remaining individuals, 17% missing single locus genotypes were present. No deviations from Hardy-Weinberg proportions were evident after Bonferroni correction (Rice 1989) in any of the samples.

Samples from the two populations from the Faroe Islands were generally polymorphic for *Pun* I (Fig. 1), except for the Faroe Bank 1992 sample, while all population samples from the Baltic Sea and the North Sea were monomorphic for the *Pun* I^A allele. Despite an apparent tendency for a reduction in the *Pun* I^B allele frequency over time in the Faroese samples, none of the changes were significant (FB78–FB92: $p = 0.10$; FB78–FB02: $p = 0.34$; FP69–FP02: $p = 0.48$). Likewise, the microsatellite analysis revealed a general pattern of temporal stability (Table 2), with very low F_{ST} values between temporal samples.

SST in June was usually warmer in the Bornholm Basin and northwestern North Sea than on the Faroe Plateau (Fig. 2; Table 3). Long-term mean temperatures in these areas were 11.4 and 10.6 °C, and therefore 2–3 °C warmer than on the Faroe Plateau (8.3 °C; Fig. 2, Table 3). Temperatures in the Bornholm Basin were more variable than those at the other sites (Fig. 2, Table 3).

There is no evidence of an overall long-term increase or decrease in temperature in the three regions (Table 3). Temperatures in two areas (Bornholm Basin and Faroe Plateau) have increased since the mid-1990s until the early 2000s; the increase is largest in the Bornholm Basin where temperatures rose 4.8 °C between 1997 and 2003 (Fig. 2). The GAM analysis explained significant multiannual temperature variability only for the Faroe Plateau (pseudo-$R^2 = 18\%$, $p <$

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0.001; $p > 0.05$ for the other regions); temperature variations at the other sites occurred at short time scales (i. e., year-to-year), which could not be resolved by the GAMs.

Discussion

Analysis of *Pan* I from historical otolith collections

Our study demonstrates that analysis of *Pan* I genetic variation can be achieved from historical otolith collections in cod, as has been demonstrated previously for microsatellites (Hutchinson et al. 2003; Poulsen et al. 2006). Compared with microsatellite analysis, the method appears to be more robust for analysis of partially degraded DNA, since high PCR amplification success was possible even for the FB69 sample, where only three microsatellite loci could be amplified. Even compared with those loci, which have an amplified fragment size similar to the *Pan* I fragment spanned by our newly designed primers, we had a higher amplification success for *Pan* I, which was almost similar to contemporary samples. A possible explanation for the different amplification success could be microsatellite-specific technical problems, such as short allele dominance and large allele dropout, causing inconsistent scoring and subsequent need to discard affected loci or individuals for further analysis. We also think the method would be well suited for even older material, since the most severe degradation of DNA from archived material appears to occur during the first years of storage (see Nielsen et al. 1999). In short, we think that the method described here is very robust and serves as a highly reliable, low-tech alternative to other *Pan* I genotyping methods (Fevolden and Pogson 1995; Case et al. 2005 and Stenvik et al. 2006), not only for historical tissue but also, for example, for real-time analysis onboard research vessels.

Evolutionary drivers of *Pan* I allele frequencies

The present study demonstrates that the *Pan* I locus has not been subject to any major changes in allele frequencies in the populations sampled over the last three to seven decades. This is consistent with neutral expectations based on our microsatellite analysis, showing similar limited genetic differentiation over time. The previously published results of cod from the North Sea and the Baltic Sea (Poulsen et al. 2006) are corroborated by the new results from the Faroe Islands in suggesting "large" effective population sizes in cod. By large, we do not necessarily mean large compared with census size, but large enough to render random genetic drift an evolutionary driver of low importance (see Poulsen et al. 2006 for a more detailed discussion on effective population sizes in cod).

Geographic comparison of *Pan* I^A frequencies with SST shows that the temperature at which *Pan* I^A frequencies begins to increase is about 6–7 °C; at lower temperatures, this allele was rare, whereas at temperatures >10 °C, *Pan* I^A frequency was nearly 100% (Case et al. 2005). In our study, temperatures at no time during the last 70 years in any of our areas reached the low temperatures that are typical of habitats occupied by cod populations dominated by very high frequencies of the *Pan* I^B allele. Generally, we observed very low frequencies of this allele in all of our samples, regardless of when or where the samples were collected. Our

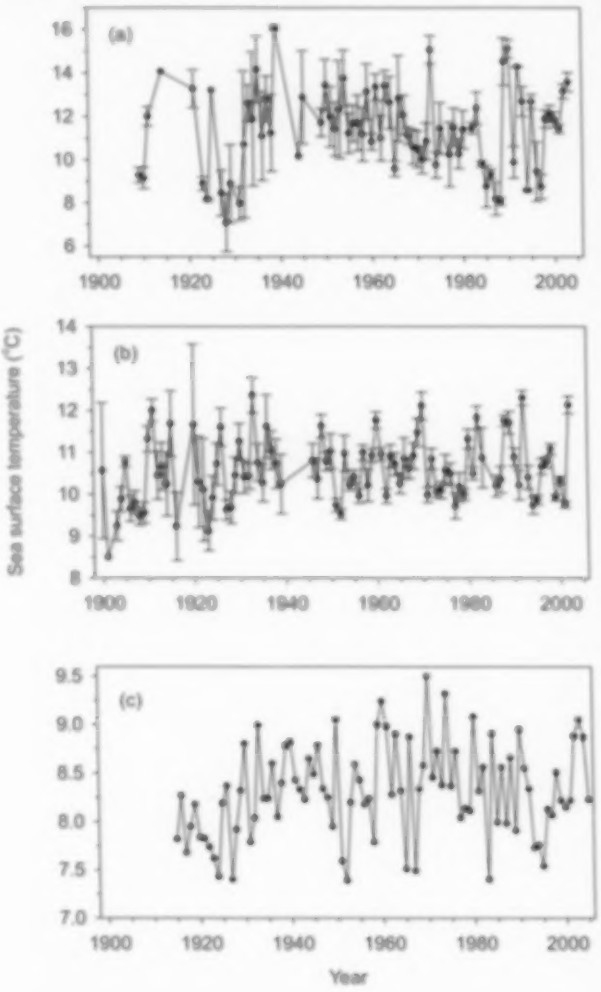

Fig. 2. Temporal development of June sea surface temperatures (mean ± two standard errors) for the Baltic Sea (Bornholm Basin), northwest North Sea, and Faroe Plateau. Means, variances, and results of linear regression analyses are available in Table 3.

results, when considered together with those of Case et al. (2005), demonstrate that the temporal variation in *Pan* I allele frequencies is much lower than the geographic variation, at least in the populations sampled and under the given historic temperature variability observed in and among our sites. However, given that temperatures in many areas of the northeast Atlantic, including the Faroe Plateau, North Sea, and Baltic Sea all have increased in the last 10–15 years (B.R. MacKenzie and D. Schiedek, unpublished data) and are expected to increase further because of future global warming (IPCC 2001; BACC 2006), *Pan* I^A frequencies will likely remain high in these regions during the coming decades given that temperature is actually an important factor determining allele frequencies at the *Pan* I locus. Both the results of Case et al. (2005) and Pampoulie et al. (2006) also suggest a correlation between *Pan* I allele frequencies and depth, implying that other factors than temperature per se

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Table 3. Summary statistics and results of linear regression of June sea surface temperature measured at three cod spawning areas in the northeast Atlantic.

Region	Latitude, longitude	*N*	Mean	Variance	R^2	*p*
Bornholm Basin, Baltic Sea	55.9°N–54.9°N, 16.3°E–14.7°E	75	11.4	3.7	0.00	0.60
Northwest North Sea	55°N–60°N, 5°W–0°	91	10.6	0.6	0.04	0.05
Faroe Plateau	62.097°N, 7.672°W; 62.116°N, 7.170°W	91	8.3	0.2	0.05	0.04

may be important for shaping allele frequencies at this locus. However, as temperature is also highly correlated with depth, disentangling direct and correlated effects may prove difficult.

The absence of a significant multidecadal June temperature increase does not allow us to come to any clear conclusions regarding the relative importance of contemporary temperature selection on shaping the distribution of *Pan* I allele frequencies as suggested by Case et al. (2005). However, demonstration of selection in the wild is difficult (Endler 1986), in particular when many environmental clines are correlated (Sarup et al. 2006) and when the specific causes of mortality are poorly known. Even if temperature is the driver for allele frequency differences, other aspects than mean June surface temperatures (e.g., maximum and minimum) could prove more important, particularly if recruitment in some years is determined after the larval stage. For example, processes occurring after settlement to the benthos can affect mortality rates during the juvenile stages (Heath and Gallego 1997), and carrying capacity for juveniles in most cod populations in the northeast and northwest Atlantic has been shown to be correlated with bottom temperature in juvenile habitat (Myers et al. 2001). Still, we see evidence of year-to-year fluctuations in temperature, which could explain why Case et al. (2005) in 1997 observed a lower frequency of the *Pan* I^A allele at the Faroe Plateau than we did (0.88 versus 0.95–0.97, *p* values of 0.023 and 0.020, respectively), also supporting the hypothesis that short-term variation in allele frequencies, as observed by Karlsson and Mork (2003), may be caused by fluctuating contemporary temperature selection (or that samples were taken at different depths). This could suggest that the polymorphism at *Pan* I observed in many cod populations are maintained by fluctuating directional selection regimes as suggested by Hedrick (2002) for major histocompatibility complex genes. However, if relatively large changes in allele frequencies caused by fluctuating selection are common, there is a very high chance that one of the alleles could be lost over time, leading to fixation. Alternatively, the observed pattern of *Pan* I genetic differentiation in cod could be explained by a combination of gene flow and contemporary directional or fluctuating selection, explaining why the most northerly cod populations from the Barents Sea are not completely fixed for the *Pan* I^B allele (see e.g., Case et al. 2005). Also, in relation to the results of this study, the few individuals with *Pan* I^B alleles found around the Faroe Islands could be migrants or descendants from migrants (hybrids) from the proximate population at the Iceland–Faroese Ridge, which according to Case et al. (2005) has a very high frequency of *Pan* I^B alleles. This would also explain the occurrence of the unexpected *Pan* I^B homozygotes in our samples. Such a gene flow selection hypothesis for the Faroe Plateau could be investigated by including samples from populations potentially donating migrants. Individuals or proportions of an individual's genotype could then be assigned to population of origin using microsatellite analysis (see Pritchard et al. 2000), and the potential migrant origin of *Pan* I^B genotypes could be determined.

Application of *Pan* I for GSI

Our findings of temporal stability of *Pan* I allele frequencies over several decades illustrate that this locus can be used as a reliable genetic marker to distinguish between cod populations from the northern and southern part of its eastern distributional range in the Northeast Atlantic and most likely also among well-defined genetically isolated cod populations on a smaller geographical scale. However, using *Pan* I as a single genetic marker on a microgeographical scale cannot be recommended before more knowledge on the relative importance of different contemporary evolutionary factors affecting this locus has been generated. Accordingly, the temporal and spatial dynamics in other regions, preferably with a higher degree of polymorphism, should be investigated using a combination of noncoding genetic markers and *Pan* I, including information on potential drivers of evolution.

The *Pan* I locus has not only been used to identify local cod populations, but also to investigate the origin of individual cod in mixed aggregations (Berg et al. 2005). However, compared with results obtained by microsatellites (e.g., Nielsen et al. 2001), this locus alone has less power for individual assignment. Instead, combining information from a number of loci subject to selection is expected to provide extreme power for individual assignment. Until now, only a few genes likely to be under selection have been identified in cod (Anonymous 2006); however, with the initiation of large-scale cod genomic projects such as the CGP (Atlantic cod Genomics and Broodstock Development), the knowledge and sequence information of genes under selection is expected to grow tremendously in the near future. Still, careful evaluation of the temporal stability of other selected genes should be conducted before they can be applied for GSI.

Pan I in cod in the southeastern part of the species distribution

The pattern of temporal genetic differentiation at the *Pan* I locus in cod from the southeastern part of the distributional range shows stability over the sampled period. There is no evidence that the apparently cold-adapted *Pan* I^B allele has been present in high frequencies in North Sea and (or) Baltic Sea cod populations for many decades. Similarly, there is little evidence of a recent reduction in *Pan* I^B allele frequencies in cod populations at the Faroe Plateau and the Faroe

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Bank. The pattern of stability is apparently caused by the very limited genetic drift (large effective population sizes) in the four populations, elucidated by the temporal stability in microsatellite allele frequencies. Similarly, there is no clear evidence of a dramatically changed temperature selection regime with respect to mean June temperatures, which potentially could have given rise to a selective sweep at *Pan* I. This illustrates that cod populations in the southeastern part of its distributional range are — and have been for a long time — characterized by a very high frequency of the *Pan* I^A allele, even though we do not have sufficient power to be able to test our initial hypothesis of a relationship between increasing temperatures and *Pan* I allele frequencies. Accordingly, *Pan* I should be considered a reliable marker for GSI in cod on a macrogeographical scale, while on a smaller geographical scale a better understanding of the evolutionary drivers and their temporal dynamics are required. To elucidate these associations, more genetic studies on cod in time and space, simultaneously applying genetic markers with different evolutionary properties, should be conducted as well as more common garden experiments of the effect of genotype on fitness at different temperatures. We expect that genes under selection, such as *Pan* I, will be the preferred high resolution markers for GSI in the future.

Acknowledgements

The project has been carried out in the frame of the EU-funded project METACOD (Grant No. Q5RS-2001-00953), and we thank Nina Poulsen, Daniel Ruzzante, Peter Wright, Fiona Gibb, and Gudrun Marteinsdottir and the rest of the project team for providing samples and stimulating discussions. We are also grateful to the EU MARBEF Network of Excellence "Marine Biodiversity and Ecosystem Functioning" (contract No. GOCE-CT-2003-505446). We thank ICES for providing hydrographic data.

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PERSPECTIVE / PERSPECTIVE

Explicit consideration of uncertainty in Great Lakes fisheries management: decision analysis of sea lamprey (*Petromyzon marinus*) control in the St. Marys River

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Abstract: We used decision analysis to assist the Great Lakes Fishery Commission (GLFC) with sea lamprey (*Petromyzon marinus*) control in the St. Marys River, which connects Lake Superior to Lake Huron and until recently has been a major source of sea lamprey infestation of Lake Huron. The process involved decision-makers, managers, and experts in an analysis of management options, objectives, and critical uncertainties affecting the expected performance of alternative choices. We developed quantitative assessments of uncertainty about sea lamprey demographics and about the implementation effectiveness of three pest control options: trapping adult lampreys, sterile male releases, and lampricide treatment. We used a stochastic simulation model to forecast future parasitic sea lamprey abundance in Lake Huron for 10 management options. Management options that included enhanced levels of trapping and sterile male releases exhibited the best performance for all measures. Our analysis substantially influenced the GLFC strategy for the St. Marys River, providing an example of how explicit consideration of uncertainty using decision analysis can influence fishery management decisions.

Résumé : Nous utilisons l'analyse décisionnelle pour aider la Commission des pêcheries des Grands Lacs (GLFC) à contrôler la grande lamproie marine (*Petromyzon marinus*) dans la rivière St. Marys qui relie le lac Supérieur au lac Huron et qui, jusqu'à récemment, était une source majeure d'infestation des grandes lamproies marines au lac Huron. Le processus réunit les décideurs, les gestionnaires et les experts pour une analyse des options de gestion, des objectifs et des incertitudes critiques qui affectent la performance attendue des divers choix. Nous avons mis au point des évaluations quantitatives de l'incertitude relative à la démographie de la grande lamproie marine, ainsi qu'à l'efficacité de la mise en oeuvre de trois choix de lutte contre ces ravageurs, soit le trappage des lamproies adultes, la libération de mâles stériles et le traitement au lampricide. Nous utilisons un modèle de simulation stochastique pour prédire l'abondance future des grandes lamproies marines parasites au lac Huron en fonction de 10 options de gestion. De toutes les options de gestion, celles qui incluent une augmentation du trappage des adultes et de la libération des mâles stériles connaissent les meilleures performances. Notre analyse a influencé de façon importante la stratégie de la GLFC dans la rivière St. Marys; c'est là un exemple qui montre comment la prise en considération explicite de l'incertitude à l'aide d'une analyse décisionnelle peut influencer les choix de gestion des pêches.

[Traduit par la Rédaction]

Received 7 October 2006. Accepted 7 June 2007. Published on the NRC Research Press Web site at cjfas.nrc.ca on 12 October 2007. J19585

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 doi:10.1139/F07-114 © 2007 NRC Canada

Introduction

Uncertainty is inherent in all natural resource management decisions. Within fisheries management, calls to take uncertainty and risk into account in decision-making have become commonplace (e.g., Walters 1986; Hilborn et al. 1993; Rosenberg and Restrepo 1994). Providing fishery managers with a quantitative evaluation of the potential consequences of alternative management actions is a primary task faced by stock assessment scientists (National Research Council Committee on Fish Stock Assessment Methods 1998). Fisheries management problems typically involve a complex system of biological and socio-economic objectives and constraints. Because of this, Lane and Stephenson (1998) contend that fisheries management should move toward implementing integrated decision-making systems in which uncertainty is explicitly considered. When uncertainty is ignored or accounted for in an arbitrary fashion, suboptimal decision options may be selected, leading to outcomes such as unnecessary losses in yields or collapse of fish stocks (Frederick and Peterman 1995). Achieving fisheries management goals is more likely when uncertainties are acknowledged, quantified, and accounted for when decisions are made.

Formal quantitative decision analysis, a well-established method of structured decision-making drawn from business (Raiffa 1968; Keeney 1982; Clemen and Reilly 2001), explicitly takes uncertainties into account when evaluating management options. In fisheries management, uncertainty can affect both the forecasted consequences of decisions and the ranking of management options (e.g., Walters 1977, 1986; McAllister and Kirkwood 1998; Peterman et al. 1998). For example, large uncertainty in estimates of recruitment rate parameters for west coast United States groundfish strongly affects how successful specific management regimes will be at achieving recovery of depleted populations (Punt and Methot 2005).

Many decision analyses have been developed in conjunction with decision makers and stakeholders to help inform decision-making processes, but results have been mixed in terms of actually influencing management decisions. Peters and Marmorek (2001) and Peters et al. (2001) describe an extensive decision analysis of options for recovering endangered Chinook salmon (*Oncorhynchus tshawytscha*) stocks in the Columbia River, USA. That effort successfully synthesized information and encouraged stakeholders to collaborate, but the recommended actions derived from the decision analysis were not implemented in part because of a lack of support from senior staff at critical agencies. By contrast, decision analyses and the associated extensive simulations of uncertainties have been crucial to a wide range of fisheries worldwide. Examples include management of the South African pelagic fishery on anchovy (*Engraulis capensis*) and sardine (*Sardinops sagax*) (Butterworth et al. 1997), baleen whales (*Balaenoptera* spp.) (Kirkwood 1997), and Australia's south east fishery (Punt et al. 2002). As well, choices about appropriate mesh sizes in commercial fisheries for Atlantic cod (*Gadus morhua*) in the Baltic Sea were influenced by consideration of uncertainties about environmental effects on growth and recruitment (Kuikka et al. 1999). Decision analyses also have played an important role in choosing designs for large-scale experimental manipulations of fisheries for groundfish (Sainsbury et al. 1997) and for leopard coralgrouper (*Plectropomus leopardus*) in Australia (Punt et al. 2001).

Unfortunately, the growing number of applications of decision analysis to management of marine and anadromous fishes worldwide is not paralleled by analogous work in freshwater fisheries; applications that have influenced management decisions have been infrequent in North America. In this paper, we apply decision analysis to a major problem confronting freshwater fishery managers in the Great Lakes region of North America: control of the exotic sea lamprey (*Petromyzon marinus*). Invasions of aquatic nuisance species are currently recognized as among the greatest threats to ecosystems such as the Great Lakes (Ricciardi 2001; Pimentel 2005); the sea lamprey is one of the best-known and most-destructive examples (Christie and Goddard 2003). Our decision analysis focused on selecting options for sea lamprey control in the St. Marys River — the channel connecting Lake Superior and Lake Huron that was the largest single source of sea lampreys in the Great Lakes in the 1990s (Schleen et al. 2003). Herein, we describe the problem faced in the St. Marys River, outline our decision analysis steps, and then document how the Great Lakes Fishery Commission (GLFC), the agency responsible for implementing sea lamprey control, responded. We show that explicit, quantitative consideration of uncertainty altered the perspective of decision makers in unexpected ways, leading to a control strategy that likely differed from one that would have resulted from a less explicit consideration of uncertainty. Finally, we reflect on the reasons for these results and offer recommendations for future applications of decision analysis to fishery problems.

Context

Sea lampreys invaded the upper Great Lakes in the middle of the 20th century and contributed to a drastic decline (extirpation in Lake Michigan) of lake trout (*Salvelinus namaycush*) populations (Smith and Tibbles 1980). Since the late 1950s, the GLFC has led a program of sea lamprey control that has suppressed sea lamprey production in nearly all Great Lakes tributaries. By far the most prominent exception has been the St. Marys River (Morse et al. 2003), the connecting channel between Lakes Superior and Huron. The large size and flow of this river, together with its role as a major shipping channel, made conventional control tactics (whole-stream lampricide applications, low-head barrier dams) cost prohibitive or impossible. Until 1999, sea lampreys produced from the St. Marys River led to wounding and associated mortality rates for several managed species (e.g., lake trout and lake whitefish (*Coregonus clupeaformis*)) in northern Lake Huron that far exceeded levels specified in fish community management objectives (Desjardine et al. 1995; Morse et al. 2003). The large number of sea lampreys produced by the St. Marys River was viewed as a key impediment to achieving these objectives.

As a consequence, sea lamprey control in the St. Marys River became a major focus of the GLFC, the binational governmental organization formed by treaty convention in 1955 to direct sea lamprey control in the Great Lakes. Dur-

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ing the 1990s, the GLFC's agents for sea lamprey control (Fisheries and Oceans Canada and United States Fish and Wildlife Service) were directed to assess sea lamprey production in the St. Marys River and develop a strategy for control (Schleen et al. 2003). A control strategy was developed based on an exhaustive survey of larval sea lampreys in the river over 4 years and continuing assessments of numbers of adult lampreys migrating into the river to spawn (Fodale et al. 2003; Schleen et al. 2003). The strategy considered new information about control options, including a new formulation of the bottom-release lampricide, granular Bayluscide[4] (Schleen et al. 2003); dye and modeling studies of the application of the liquid lampricide 4-nitro-3-(trifluoromethyl)phenol (TFM) (Shen et al. 2003); preliminary deployment of new traps to capture adult lampreys; and full-scale deployment of the sterile male release technique (SMRT: Twohey et al. 2003).

In 1997, the GLFC adopted the strategy, which comprised an integrated program of annual trapping of adults and release of sterilized males and a one-time application of Bayluscide. As part of this program, traps were placed in the river during the spring spawning migration and were visited daily, with captured sea lampreys destroyed (females) or used for sterile male releases (males). During the 1990s, trapping was estimated (by a mark–recapture method) to remove about 45% of the annual spawning run. Male sea lampreys collected from traps operated throughout the Great Lakes (the majority was obtained from the St. Marys River) were chemically sterilized and released into the St. Marys River. The number released was expected to produce a 4:1 ratio of sterile to nonsterile males, effectively reducing reproduction by the untrapped spawners by an additional 80% (Twohey et al. 2003). The one-time application of lampricide was applied sequentially to different portions of the river during 1998, 1999, and 2001 and in total led to treating 981 ha of high-density larval habitat in the river, estimated to contain roughly 75% of the total larvae in the river.

The decision to adopt this strategy was based on deterministic models presented to the GLFC that forecasted the effects of various management options on sea lamprey and lake trout populations along with a cost–benefit analysis of those management options (Schleen et al. 2003). Preliminary evidence suggests that the actions taken during 1998–2001 have resulted in significant declines in sea lamprey abundance in the St. Marys River and subsequently in northern Lake Huron (Adams et al. 2003; J. Adams, US Geological Survey, Great Lakes Science Center, 1451 Green Road, Ann Arbor, MI 48105, USA, unpublished data).

The control strategy recommended and adopted in 1997 was not designed, nor intended to be, a long-term strategy. Instead, its objective was to severely reduce the St. Marys River sea lamprey population. The strategy represented the GLFC's most intensive attempt to use trapping and SMRT to suppress lamprey production in a river and was consistent with its vision for reducing lampricide use (GLFC 1992). This strategy included a one-time Bayluscide treatment of high-density habitat in the river. All other substantial sea lamprey-producing streams in the Great Lakes basin receive regular application of lampricides, typically on a 3- to 5-year rotation, before the larval populations that reestablish after treatment can metamorphose and migrate to the lakes. The lampricide treatment of the St. Marys River during 1998–2001 was very expensive. The annualized cost was about US$1.0 million, compared with an annual sea lamprey control budget for the entire Great Lakes basin of approximately US$5.3 million. The annual cost for the St. Marys River trapping and sterile male release options that were implemented at the same time was roughly US$0.5 million. The three-pronged treatment program was judged necessary in 1998–2001 because of the perceived large, but unquantified, uncertainty about how effective less costly control actions (trapping and sterile male releases) would be if implemented alone. An expensive long-term control strategy using lampricides, however, would redirect limited funds from controlling other lamprey-producing rivers in the Great Lakes basin. Consequently, the GLFC faced the challenge of developing a feasible long-term strategy to maintain effective control in the St. Marys River. We were aware of the need for a longer-term approach, and knowing that decision analysis could provide a process for formally considering the uncertainties that made development of an optimal strategy so difficult, we decided to use sea lamprey control of the St. Marys River as a case study for application of decision analysis in the Great Lakes.

Conducting the decision analysis

To conduct our decision analysis, we followed the eight steps of decision analysis described by Peterman and Anderson (1999). Herein, we organize our description of the St. Marys River sea lamprey decision analysis around these eight steps. We held three workshops with managers, scientists, and other stakeholders between 1999 and 2001 to develop the decision analysis and then presented and discussed our findings with the GLFC on four occasions during 2001–2002, leading to the decisions reported below.

Identify alternative management actions

We first convened an initial meeting of scientists, agency management personnel, and other stakeholders to explain our decision analysis approach and identify a list of alternative sea lamprey control actions. The group identified trapping, SMRT, and application of Bayluscide to specific locations as the primary actions. There was considerable interest in examining various combinations of levels and timing of these actions. Additional or improved adult traps could be deployed to increase the proportion of the spawning population captured and removed as they migrate upstream to spawn. Because SMRT relies on trapping animals from the St. Marys and other Great Lakes tributaries, and most animals come from the St. Marys, higher trap catches lead to more males being available for SMRT. Therefore, the group believed that different trapping intensities and SMRT should be examined in concert with each other. The costs of these options were low relative to lampricide application and did not include costs associated with the large capital investment of the traps

[4] Bayluscide is the commercial name for 2′,5-dichloro-4′-nitrosalicylanilide, also known as niclosamide. Development of a granular formulation enabled the use of this lampricide for application to specific patches of larval habitat rather than to the entire river, as is required for conventional lampricide applications.

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Table 1. Management options considered by the Great Lakes Fishery Commission for integrated control of the sea lamprey (*Petromyzon marinus*) population in the St. Marys River.

Option	Description	Target trapping rate	Target SMRT ratio	Target kill rate	Actual kill rate	Annualized cost (US$)
1	No control	0	0	0	0	0
2	Trapping only	0.4	0	0	0	59 000
3	Enhanced trapping	0.7	0	0	0	134 000
4	Trapping + SMRT	0.4	4	0	0	555 000
5	Enhanced trapping + SMRT	0.7	7	0	0	630 000
6	Bayluscide only; 108 ha	0	0	0.15	0.093	121 250
7	Trapping + SMRT + Bayluscide; 108 ha	0.4	4	0.15	0.093	676 250
8	Enhanced trapping + SMRT + Bayluscide; 108 ha	0.7	7	0.15	0.093	751 250
9	Trapping + SMRT+ Bayluscide; 260 ha	0.4	4	0.25	0.176	787 000
10	Trapping + SMRT + Bayluscide; 812 ha	0.4	4	0.34	0.277	1 467 500

Note: All rates are expressed as proportions. Trapping rate is the proportion of the spawning run captured by traps. SMRT (sterile male release technique) ratio is the ratio of sterile to fertile males in the spawning population. The target kill rate refers to the expected kill rate if the true larval population distribution is identical to that observed during the 1993–1996 survey. The actual kill rate adjusts for the effect of uncertainty in the true spatial distribution. Bayluscide treatments were applied once every 4 years.

currently in place nor of the existing sterilization facility. Because of the large fixed capital investment compared with the smaller annual operating costs, only options where trapping and sterilization were done every year were considered. Bayluscide applications are independent of both trapping and SMRT; therefore, they could be investigated at various intensity levels without defining a relationship between Bayluscide and the other control actions. In considering Bayluscide treatments, it was assumed that the river would be treated every 4 years, corresponding to the development time of larval sea lampreys. Treating the river with TFM was briefly discussed, but the group concluded that this potential action was cost-prohibitive given the extremely large volume of water in the St. Marys River. TFM costs were estimated to be at least three times as great as those for Bayluscide to achieve a comparable reduction in larval sea lampreys.

In 2002, the Sea Lamprey Integration Committee, a policy advisory committee to the GLFC made up of fishery and sea lamprey program managers and experts, developed a subset of 10 management options for consideration by the GLFC (Table 1). We compare these options in this paper. The options consisted of various combinations of trapping, sterile male release ratios, and Bayluscide applications. The trapping and SMRT options included continuation of current target levels (40% trapping and a 4:1 ratio of sterile to nonsterile males) and an enhanced option (70% trapping and a 7:1 ratio) based on the judgment of program staff about the maximum levels of trapping and SMRT that could be achieved. Cost estimates for each of the management options (Table 1) were obtained by consulting with sea lamprey control agents and were annualized over a 30-year period.

Specify management objectives

Defining the set of management objectives proved more difficult for the group. Ultimately, they agreed that controlling sea lamprey populations in the Great Lakes provides the opportunity for a healthy, productive fish community. Restoring lake trout in northern Lakes Huron and Michigan is a particular concern, and therefore some believed that self-sustaining lake trout populations should be a management objective. However, commercial and recreational fishing, stocking levels, and the lack of natural spawning populations all affect lake trout populations, in addition to sea lamprey control. The group concluded that while restoring lake trout was indeed a management goal, too many external factors play a part in lake trout restoration for it to be an appropriate management objective for a decision analysis concerned with St. Marys River sea lamprey control.

The group agreed instead to use an objective of reducing the number of parasitic sea lampreys in Lake Huron, arguing that reducing the number of sea lampreys is critical to lake trout recovery efforts in Lake Huron. Clearly, management objectives must be specified in explicit, quantitative terms to be useful in a decision analysis (Peterman and Anderson 1999). Thus, in addition to the target reductions in parasitic sea lamprey abundance, the group's management objective also considered the timing of reductions (how quickly benefits of control will be realized) and the probability of achieving a specified (target) level of reduction. As well, recent agreements negotiated among the State of Michigan, several Native American tribes, and the US Federal government implicitly established target levels for future sea lamprey abundance in Lakes Huron and Michigan (US v. Michigan 2000) that could be used to compare simulated performances of different management options. These latter targets were based on the same deterministic simulations of likely effects of sea lamprey control that were used to inform the 1997 program decisions discussed above, together with a determination that such reductions would be compatible with lake trout rehabilitation objectives. Finally, because greater reductions in sea lampreys were generally associated with greater costs of control, the group also recommended developing an economic basis for defining benefits of reduced parasitic abundance (cf. Koonce et al. 1993), such that management options could be compared based on their net economic benefit.

Identify the uncertain states of nature

Management decisions are invariably made in the presence of many uncertainties, but explicitly including all of them in a decision analysis is impractical. Initially, it can seem like a daunting task to narrow the list of possible uncertainties to a small number of key ones that dominate the decision process. However, it has been our experience that

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groups of experts are able to quickly arrive at such a list, and this was the case for the St. Marys River sea lamprey problem. The group identified two principal uncertainties. The first was uncertainty in St. Marys River sea lamprey population dynamics and particularly the stock–recruitment relationship. Little was known about sea lamprey demographic rates, their productivity, and their degree of compensation as abundance increases (i.e., larval density-dependent mortality). This uncertainty made it impossible to forecast with confidence the effects of control options that seek to reduce reproductive success (e.g., trapping and SMRT) on future sea lamprey abundance. The group also thought that implementation uncertainty was critical; how effective will each control option be at actually reducing the abundance of a targeted life stage? For Bayluscide applications, the targeted life stage is larvae. During 1993–1996, nearly 12 000 locations in the St. Marys River were sampled to determine the spatial distribution of larvae and thereby identify specific locations for targeting lampricide applications. Because repeating this intensive mapping effort each year is impractical, considerable uncertainty will exist about the future distribution of larvae in the river, which leads to substantial implementation uncertainty about the effectiveness of a Bayluscide treatment. Similarly, for trapping and SMRT options, there will be a deviation between the target level of control (percentage of population trapped, sterile male to nonsterile male ratio) and the actual level achieved in a particular year. Thus, the uncertain states of nature that we focused on in our decision analysis concerned demographic parameters for the sea lamprey population and the effect of each control option on the abundance of the targeted life stage.

Assign probabilities to the states of nature

Methods for assigning probabilities to the states of nature include from using subjective human judgment when little or no data are available, using standard classical statistical methods when empirical data are available, or using Bayesian methods when prior information is also available (Morgan and Henrion 1990). Within this decision analysis, we utilized a variety of methods to assign probabilities to the uncertain states of nature, considering the quantity and quality of the data available for each. To quantify uncertainty about sea lamprey demographic parameters, we used an approach similar to statistical catch-at-age analysis (Fournier and Archibald 1982). We developed a Bayesian age-structured population model that reflected the life history of St. Marys River sea lampreys and used six sources of data to estimate the parameters of this model (Haeseker et al. 2003). Those six data sources were a catch-per-unit-effort lamprey abundance index based on incidental commercial catches, mark–recapture population estimates for the number of spawning-phase St. Marys River sea lampreys, mark–recapture population estimates for the total number of Lake Huron sea lampreys, length–frequency data on larval sea lampreys in the St. Marys River sampled using Bayluscide, length–frequency data on larval sea lampreys in the St. Marys River sampled using electrofishing, and age composition data on metamorphosing sea lampreys from the St. Marys River. Parameters estimated in our analysis included time series of age-0 abundance from 1967 to 1996 (recruits), initial age composition in 1966, a constant larval natural mortality rate, two parameters describing the probability of larval metamorphosis as a function of age, and a parameter describing the proportion of metamorphosed juvenile sea lampreys that survive to maturity and return to the St. Marys River to spawn. We obtained point estimates for these parameters by maximizing the joint likelihood of the six data sources, with each data source being weighted relative to its empirical variance estimate (Haeseker et al. 2003).

We used Markov chain Monte Carlo (MCMC) methods to obtain 1000 samples from the approximate joint posterior distribution of the complete set of demographic parameters. Using the method of composition (Tanner 1996, pp. 52–54), we then augmented each of these samples with sampled values of the parameters of a Ricker stock–recruitment relationship. These Ricker parameters were determined based on the demographic parameters, which included a time series of recruitment and parameters from which a time series of spawners can be calculated. Our approach to obtaining the sample of Ricker parameters was as follows. First, for each of the 1000 sets of demographic parameters, we fit the linear form of the Ricker model

$$(1) \qquad \ln(R/S) = \ln(\alpha) - \beta S + \varepsilon$$

to the time series of numbers of age-0 larvae (R) and numbers of female spawners (S) that produced R. Here the error term was normally distributed, $\varepsilon \sim N(0, \sigma^2)$; and α, β, and σ^2 are the parameters about which we need to incorporate uncertainty. The maximum likelihood estimates of these regression parameters are the most likely values given the corresponding demographic parameters. Rather than using them directly as our sample of the stock–recruitment parameters, we recognized that other Ricker parameters are also plausible given the demographic parameters. We therefore obtained each sample of Ricker parameters by randomly selecting α and β from a bivariate normal distribution, with mean equal to the values of α and β estimated by linear regression, and variance–covariance matrix also taken from the regression results. We selected σ^2 from a scaled inverse χ^2 distribution, with mean equal to the regression point estimate (Gelman et al. 1995, p. 480). In addition to the sampled stock–recruitment parameters characterizing the uncertainty within each sample set of demographic parameters, our approach of using 1000 plausible sets of demographic parameters (and hence time series of stock and recruitment) characterized the uncertainty in the stock–recruitment series arising from measurement error in the observed data. We did this because ignoring measurement error can result in biased stock–recruitment parameter estimates (Ludwig and Walters 1981). These methods are described in detail in Haeseker et al. (2003).

We also developed probability distributions for characterizing uncertainty in the effectiveness (i.e., implementation uncertainty) of the three types of control measures. To quantify implementation uncertainty about the effectiveness of Bayluscide treatment, we developed a stochastic simulation model of larval abundance in the St. Marys River. We began with two spatially referenced data sets containing information on larval distribution and abundance. The first we refer to as the index data set, which is based on annual samples

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collected from small (35–165 ha) index sites in the river over 5 years (1994–1998). The other we refer to as the river-wide data set, which was based on an extensive, systematic sampling of all larval habitat in the river over 4 years (1993–1996: Fodale et al. 2003). We used these data to estimate the proportion of larvae that would be killed by a particular spatial distribution of Bayluscide granules. Specifically, we used the index data set to estimate the parameters of a two-stage, generalized linear model (Haeseker 2001), which determines the probability distribution of abundance at individual sample locations. The probabilities were a function of habitat quality at those locations, the measured abundance at those locations the previous year, and the inverse distance-weighted average of abundance at neighboring locations. The first stage modeled the probability of presence (non-zero catch in the samples) of larvae using a binomial link function. The second stage modeled the probability distribution of actual catches, conditional on a non-zero catch, using a gamma link function. We then stochastically applied this model to the spatial distribution of larval abundances from the river-wide data set to generate a sample of 1000 maps representing possible distributions and abundances of larvae in 1999, the year of the major treatment of the St. Marys with Bayluscide. For each map, we calculated the proportion of larvae that would have been killed given the actual spatial distribution of Bayluscide applications in 1999. The resulting probability distribution of treatment effectiveness values (proportion of larvae killed) across the 1000 maps characterized the implementation uncertainty associated with the actual 1999 Bayluscide application. We then calculated implementation uncertainty for a range of more limited Bayluscide applications (treating fewer hectares of larval habitat) by repeating this process but restricting the application of Bayluscide to the highest-density plots (areas of larval habitat) that could be treated given the spatial scale of the application. We used the results of these stochastic simulations (Fig. 1) to define probability distributions of effectiveness of Bayluscide applications for the range of management options we considered (Table 1). While these stochastic simulations likely accounted for most of the variability in larval abundance patterns, we did not attempt to account for the additional variability caused by uncertainty in the model parameters themselves.

We quantified implementation uncertainty associated with trapping efficiency (i.e., the proportion of the spawning population of sea lampreys removed by trapping) by considering the variation in observed trapping efficiency during a period of fairly consistent operation of the traps. During 1991–2000, the trapping efficiency ranged 20%–54%. Efficiency varied approximately uniformly about the mean, so we used a uniform (0.20, 0.54) distribution to represent the probability distribution of trapping efficiency given status quo effort (and a 40% target efficiency). For management options that involved increased investment in trapping, so that target efficiency would be 70%, we assumed a uniform (0.50, 0.84) probability distribution. Under this characterization, we assume that if target efficiency increases to 70%, variation about the mean will remain unchanged.

We assessed implementation uncertainty associated with sterile male releases by comparing observations of the proportion of viable embryos in nests with the expected reduction in viable embryo production given the observed ratio of sterile to nonsterile males in the spawning population. Male lampreys were collected from traps in the St. Marys River and other Great Lakes tributaries, sterilized at a central facility, and then released back into the St. Marys River (Twohey et al. 2003). A mark–recapture method was used to estimate the number of spawning-phase adult sea lampreys migrating into the river. These estimates were combined with trap capture data to estimate trap removal efficiency and thus the number of nonsterile males that successfully reached the spawning grounds above the traps. Assuming that sterile males compete effectively with nonsterile males for mates (Bergstedt et al. 2003), the ratio of sterile males to nonsterile males can be used to calculate an expected proportion of effective female spawners (females mating with sterile males will produce nonviable embryos):

$$p_{\mathrm{EFS}} = \frac{1}{1 + N_s/N_n} \tag{2}$$

where p_{EFS} is the expected proportion of effective female spawners, and N_s/N_n is the ratio of sterile to nonsterile males. For example, a ratio of 3:1 would result in a prediction that only 25% of the females would successfully spawn. Under conditions of incomplete viability and fertilization success, the observed proportion of viable embryos ($p_{\mathrm{VE}_{\mathrm{obs}}}$) would be

$$p_{\mathrm{VE}_{\mathrm{obs}}} = p_{\mathrm{EFS}}\,\bar{V} \tag{3}$$

where $\bar{V}$ is the average level of egg viability for matings between females and fertile males. In the St. Marys River, surveys of nests occupied by fertile males displayed an average

Fig. 1. The relationship between the area (ha) of the St. Marys River treated with Bayluscide and the percentage of the larval sea lampreys (*Petromyzon marinus*) population exposed to the lampricide. The actual proportion that is killed is estimated to be 75% of those exposed. The line represents the relationship when perfect knowledge of the larval distribution in the river is assumed. Each vertical cluster of points represents the results of 1000 simulations of possible larval distribution patterns for a particular area treated (to improve visibility, points are slightly spread along the *x* axis), based on a treatment strategy that targets areas assumed to have the highest densities. See Haeseker (2001) for details of simulation methods.

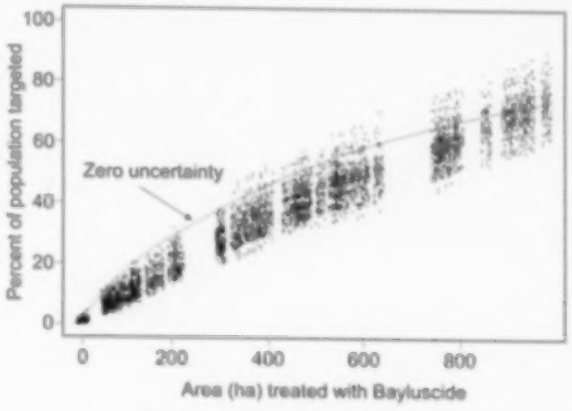

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Table 2. An explanation of symbols used in eq. 5, the simulation model used to forecast sea lamprey (*Petromyzon marinus*) dynamics.

Symbol	Description	Type
i	Age class	i
t	Year	i
l	Abundance of larvae in the St. Marys River by age and year	v
P	Abundance of parasitic lampreys in Lake Huron by year	v
S	Abundance of effective female spawners in the St. Marys River by year	v
m	Age-specific proportion of larvae that metamorphose	p
M	Annual instantaneous natural mortality rate for larvae	p
λ	Conversion of Lake Huron parasitic lampreys into St. Marys River adult lampreys (a product of survival during the parasitic phase and the fraction of the surviving population that returned to the St. Marys River)	p
α, β	Ricker model parameters	p
ε	Normally distributed recruitment process error	p
σ^2	Variance of recruitment process error	p
P_t	Parasitic lampreys from sources other than the St. Marys River	c
p_f	Proportion of adult lampreys that are females	c
B	Year-specific removal of larvae due to Bayluscide application	C
T	Proportion of lampreys removed due to trapping	C
p_{EFS}	Proportion of females that successfully mate with fertile males	C

Note: Types are as follows: i, index; v, state variable; p, uncertain parameters; c, assumed constants; C, control (policy) variables or management actions.

viability of $\bar{V} = 43.4\%$, which we used as a baseline level of reproductive success for matings between females and fertile males (Bergstedt et al. 2003).

We combined these expected reductions in effective female spawners and reproductive success with observations of actual embryo viability among nests in the St. Marys River to estimate the ratio of sterile to nonsterile males that would have been needed to yield the observed reduction in average embryo viability from the baseline level. Nest surveys to estimate the proportion of viable embryos were conducted in the St. Marys River spawning area each year for 1992–2000. We used these observations of embryo viability from the nest surveys, where the male involved in mating was unknown, to estimate the ratio of sterile to nonsterile males that would have been needed (N_s/N_n^*) to yield the observed reduction in average embryo viability from the baseline level:

$$(4) \qquad N_s/N_n^* = \frac{\bar{V}}{p_{\mathrm{VE}}\,v_{\mathrm{obs}}} - 1$$

Finally, we compared this estimate (N_s/N_n^*) to the observed ratio of sterile males to nonsterile males based on releases and mark–recapture estimates. We used the deviation between the two estimates for each year as a measure of the uncertainty associated with the implementation of the sterile male release technique. In effect, we assumed that the ratio calculated from the numbers of sterile males released was the management target and the ratio estimated from the nest surveys was a measure of the actual effective ratio that was achieved. To simulate uncertainty in performance of the sterile male release technique in the future, in each simulated year, we randomly selected (with replacement) a deviation from the expected performance from the observed set of deviations for the years 1992–2000 and applied the deviation to the predicted proportion of effective female spawners (eq. 2) associated with the level of SMRT that was being evaluated. The observed deviation from 1997 was not used because river conditions made the mark–recapture estimates unreliable (M. Twohey, US Fish and Wildlife Service, Marquette Biological Station, 1924 Industrial Parkway, Marquette, MI 48555, USA, personal communication).

Forecast outcomes of management options

We used an age-structured simulation model, similar to the demographic parameter estimation model (Haeseker et al. 2003), to forecast future abundance of parasitic sea lampreys in Lake Huron from 2001 to 2030. Whereas the parameter estimation model was used for reconstructing historic population dynamics, the simulation model was used to forecast future population dynamics while accounting for uncertainties identified above and the effects of different management options.

The model consisted of the following equations (for symbol description, see Table 2):

$$(5) \qquad l_{i+1,t+1} = l_{i,t}\, e^{-M}\,(1 - m_i)(1 - B_t)$$

$$P_{t+1} = \sum_{i=1}^{n} l_{i,t}\, m_i + P_t$$

$$S_{t+1} = P_t\, \lambda\, p_f\, (1 - T_t)\, p_{\mathrm{EFS}}$$

$$l_{0,t} = \alpha\, S_t\, e^{-\beta S_t + \varepsilon_t}$$

$$\varepsilon_t \sim N(0, \sigma^2)$$

The forecasting model began in 2000, whereas the parameter estimation model could only estimate population abundances up to 1996. We initialized the forecasting model by projecting the age composition from 1996 to 2000 using estimated demographic parameters and the actual control actions (trapping, sterile male releases, and Bayluscide applications) that occurred during this period. This initialization process was conducted for each of the 1000 sets of demographic parame-

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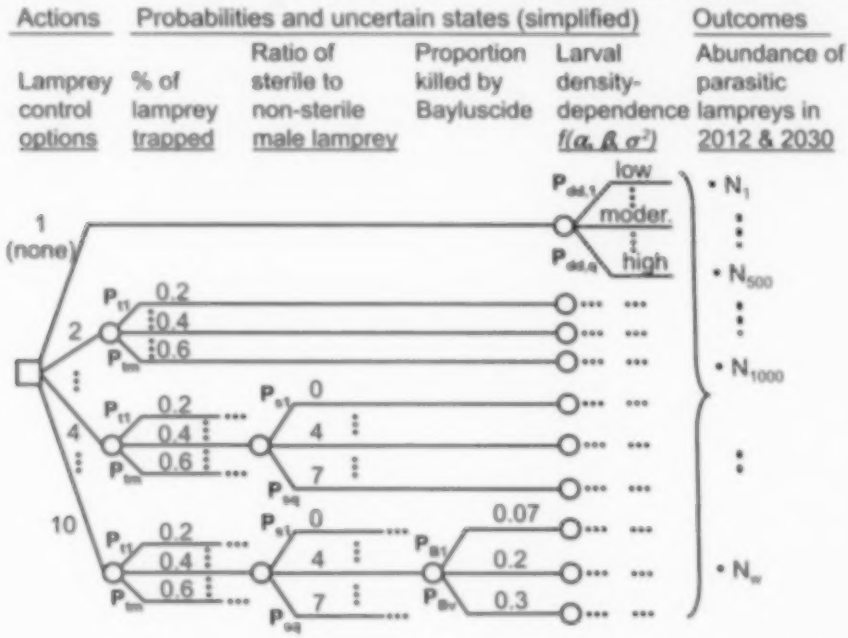
Fig. 2. A decision tree for options facing sea lamprey (*Petromyzon marinus*) managers for the St. Marys River. Lines emanating from the square node on the left represent alternative management options, which are fully defined in Table 1. Lines emanating from round uncertainty nodes represent alternative realized states of actions (for trapping, sterile male releases, and Bayluscide treatments) or alternative states of nature (for sea lamprey demographic parameters). Vertical ellipses in the control options column indicate that other options were evaluated but are not shown here because of space limitations. For alternative realized states of nature, continuous ranges of values were analyzed but are only shown here as discrete examples. Horizontal ellipses indicate that branches from uncertainty nodes are repeated as indicated elsewhere for that category or state. Probabilities on each state (P_{ij}) represent the weighting placed on each type of state i and value j. One outcome is calculated for each combination of control option and alternative realized states of actions and states of nature.



ters obtained from the MCMC procedure, which produced 1000 sets of initial conditions for the forecasting model. For each management option, we ran 100 000 simulations comprising 100 realizations across the 2001–2030 time period of the stock–recruit process error term ε_t (i.e., density-independent recruitment variation) for each of the 1000 sets of demographic parameters and their corresponding Ricker parameters.

Decision tree

The decision problem considered here can be visualized as a decision tree that depicts the management options and the critical uncertainties that may influence the expected performance of different management options (Fig. 2). Uncertainties are represented by the circular uncertainty nodes, whereas alternative management options arise from the square management options nodes. Outcomes (defined here as the time series of median abundance of the parasitic stage of sea lampreys in Lake Huron 2001–2030) are calculated for each branch arising from the uncertainty nodes and for each management option. Based on the management options under consideration, individual types of uncertainties may or may not be included in the outcome calculations. For example, one potential management option is to cease all efforts to control sea lampreys in the St. Marys River. In this case, only uncertainty about sea lamprey demographics will affect the outcomes. The other (implementation) uncertainties are only considered when the relevant management tactic is included in the option.

Rank management options

We used three performance measures to rank the outcomes of each management option. First, we computed the median forecasted abundance of parasitic sea lampreys for each year from 2001 to 2030. Second, we computed the distribution of economic net benefits for each option, based on the difference between the annualized cost of the option and the estimated economic benefit of a suppressed lamprey population. We describe our method for estimating net benefits below. Third, we computed the probability of the Lake Huron parasitic lamprey population falling below a target level of 114 000 parasites, measured as the proportion of simulations meeting this criterion. This target was derived from agency expectations for sea lamprey suppression used in negotiations among the State of Michigan, several Native American tribes, and the US federal government (US v. Michigan 2000) concerning acceptable harvests of lake trout and lake whitefish. We compared management options by examining the status of each of these performance measures in the short term (2012) and in the long term (2030).

The annualized costs of the management options we considered varied from US$0 to US$1 467 500 (Table 1). Trapping alone options were comparatively inexpensive (options 2 and 3), as was the option that included only Bay-

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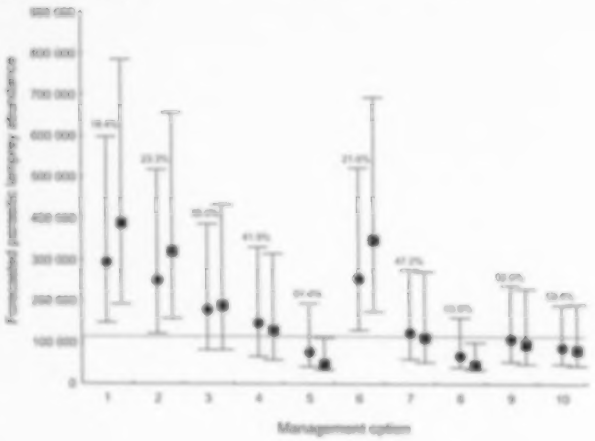
Fig. 3. Forecasted median and 25th and 75th percentiles of parasitic sea lamprey (*Petromyzon marinus*) abundance in Lake Huron in 2012 (circles) and 2030 (squares) for each of the 10 management options considered in our decision analysis. The broken horizontal line represents a parasitic abundance of 114 000. Simulation outcomes for 2012 lying below this value represent successful achievement of the management objective implied by the interagency negotiations on acceptable harvest levels (US v. Michigan 2000). Numbers at the top of error bars are percentages of simulations that successfully achieved that objective for 2012.



luscide applied to a limited area (option 6: 108 ha compared with 910 ha in 1998–2001). Options that combined trapping, sterile male releases, and Bayluscide applications were most expensive (options 7, 8, 9, 10). The costs of enhanced trapping and sterile male release (trapping at 70% efficiency, SMRT ratio of 7:1, option 5, US$630 000) were about 70% of the costs of a large-scale Bayluscide application with no trapping or sterile male methods of control (812 ha, option 10, US$912 500).

We calculated the economic benefits of sea lamprey suppression using an "implied value" approach (Stewart et al. 2003). In 1997, the GLFC selected an integrated control strategy for the St. Marys River that included a 40% rate of trapping, a 2.8 sterile to nonsterile male ratio, and treating 910 ha of larval habitat with Bayluscide. They chose this option over less expensive options that did not include Bayluscide applications (Schleen et al. 2003). The more expensive option was expected to result in a larger and more rapid decline in parasitic sea lamprey abundance. The fact that the Commission chose the more expensive option given the expected difference in outcomes of the different options implied a certain minimum benefit value for the suppression of lamprey abundance. We calculated net benefit from

$$B_i = O_i v - C_i \qquad (6)$$

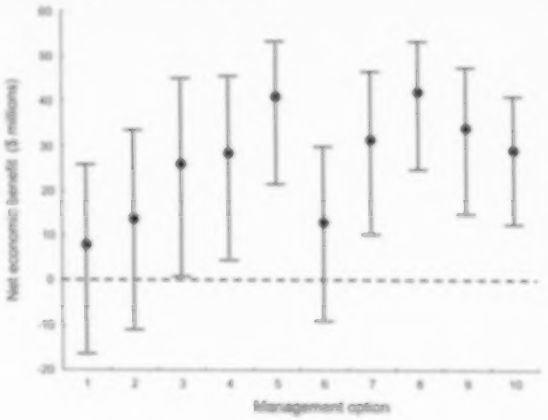
$$B_i > B_j = O_j v - C_j$$

where B_i is the net benefit of the preferred option i, O_i is the outcome of option i, v is the value of a unit change in O, C_i is the cost of option i, and j is an option less preferred by decision makers. We estimated a value v that is the minimum value to satisfy the above inequality:

$$v > (C_i - C_j)/(O_i - O_j) \qquad (7)$$

We estimated v by defining the outcome O as the discounted, 15-year total reduction in parasitic lampreys for each treatment option based on the forecasted outcomes from the deterministic model used by the GLFC in 1997. The discount rate (6%) and time horizon for these calculations was determined through consultation with GLFC staff that were involved in the 1997 decision. The value obtained from these calculations was US$15.65 per lamprey, which implies that the GLFC considered additional lampreys removed by the more expensive treatment to be worth at least this amount to justify this treatment. In this analysis, we used this implied value to calculate the discounted economic net benefit from 2002 to 2030 using the same 6% discount rate applied to both costs and benefits.

Fig. 4. Medians and 25th and 75th percentiles of the estimated net economic benefit of each of the 10 management options considered in our decision analysis.



Sensitivity analysis

We did not quantify the uncertainty associated with two important inputs to the forecasting model: the absolute contribution of parasitic sea lampreys to Lake Huron from sources other than the St. Marys River (P_i) and the assumed economic value (v) of a unit change in parasitic lamprey abundance in Lake Huron. To determine whether our conclusions were robust to our assumptions about these values, we conducted a simple sensitivity analysis for these two quantities. We repeated the simulations with values of 15 000 and 60 000 for P_i (the nominal value was 30 000) and with values of US$12 and US$20 for v (the nominal value was US$15.65); we compared the resulting rank order of the 10 management options for each set of simulations. We selected these values to represent plausible upper and lower bounds on these two model variables. In addition to examining the sensitivity of the rank order of options to these model inputs, we also examined the sensitivity to the target abundance performance measure described under "Rank management options" above.

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Results

The median parasitic population at the start of all simulations (year 2002) was 127 000. In the absence of control (option 1 in Table 1), the median population increased to 295 000 by 2012 and to 388 000 by 2030 (Fig. 3). The large interquartile range (191 000 – 784 000) for the forecasted parasitic abundance in 2030 in the absence of continued control (option 1) indicates the large uncertainty associated with the demographic parameters of the model. As expected, the mean (weighted average) net economic benefit for the no-control option was close to zero (–$0.25 million), while the median was positive ($7.9 million) because of the lognormal variation in the recruitment model, but the interquartile range overlapped zero (–$16.3 million to $26.0 million; Fig. 4).

The least expensive options — trapping alone (option 2) and Bayluscide applications alone to only a small area (108 ha, option 6) — were not effective at reducing parasitic abundance relative to the no-control option. For example, forecasted median parasitic abundance in 2030 resulting from option 2 was only 17% lower than that for no control, and option 6 was only 10% lower. Although the costs of these options were relatively low, the net economic benefits were only slightly higher than the no-control option (Fig. 4).

In terms of reducing the abundance of sea lampreys, options 5 (enhanced trapping and SMRT) and 8 (enhanced trapping, SMRT, and Bayluscide on a small area) clearly demonstrated the best performance of the nine control options considered, particularly by the year 2030 (Fig. 3). These were the only options where the upper quartiles of abundance fell below the lower quartile of the no-control option by 2030. These were the only two options considered that included enhanced trapping combined with sterile male releases (Table 1). Despite their comparatively high costs, these two options yielded the highest median net economic benefit as well, although the uncertainty in this performance indicator was greater than that for population size (Fig. 4). Increasing the area of the river treated with Bayluscide (options 9, 10) did not improve net economic benefit nearly as much as enhancement of trapping and SMRT control (options 5 and 8).

The sensitivity analysis showed that our conclusion about the rank order of management options did not depend on the choice of performance indicator or the assumed value of two parameters for which we did not specify uncertainty nodes. Options 5 and 8 still ranked highest when we based the ranking on the proportion of simulations for which forecasted parasitic abundance was less than the target level of 114 000 in 2012 (Fig. 3). For the best option (8), 65.9% of all simulations met the target, compared with only 18.4% for the no-control option (1). Increasing or decreasing either the number of parasitic sea lampreys from other Lake Huron sources (P_1) or the implied value of a unit change in sea lamprey abundance (v) also did not result in any change to the rank order of the management options based on median parasitic lamprey abundance. Rank orders based on the economic net benefit calculation changed only slightly: options 5 and 8 still ranked highest, but the most expensive option (10) had a lower ranking (7th of 10 instead of 5th of 10) when v was reduced to US$12.

We reported these results to the Sea Lamprey Integration Committee and the GLFC during the fall of 2002. The GLFC considered our analysis as they debated alternative management strategies for the St. Marys River for 2003 and beyond.

Discussion

During the period leading up to the earlier 1997 decision to treat 910 ha of the St. Marys River with Bayluscide in 1998–2001, it was widely held that the risks of relying only on suppressing reproduction with trapping and SMRT were too great. In the absence of a quantitative analysis of these risks, such as the one presented here, this view was largely based on a subjective, qualitative perception of year-to-year variability in sea lamprey recruitment. Consequently, the GLFC elected to conduct the expensive and extensive Bayluscide application at that time and even began to question the merits of relying at all on trapping and SMRT. Since that time, sea lamprey populations have declined substantially both in the St. Marys River and northern Lake Huron, and more importantly, so have wounding rates and mortality rates for host fishes in Lake Huron (J.V. Adams, US Geological Survey, Great Lakes Science Center, 1451 Green Road, Ann Arbor, MI 48105, USA, unpublished data).

During winter 2002–2003, the GLFC considered this "either-or" scenario (of using trapping and SMRT or not), and using our decision analysis as a guide, the GLFC decided to select the combined enhanced trapping, SMRT, and limited Bayluscide application (option 8). They decided to invest in enhanced trapping and SMRT control, primarily through the deployment of additional traps in the St. Marys River. They decided to treat 87 ha of the St. Marys River with Bayluscide during summer 2003 and to consider further Bayluscide treatments of the river annually, based on larval population surveys. During 2004–2006, 89, 144, and 107 ha of the river, respectively, have been treated with Bayluscide. They endorsed an ongoing program of monitoring larval populations in the river, using an adaptive sampling strategy that allowed both statistically valid estimates of larval population size in the river and some measure of the spatial distribution of larvae throughout the river. To maintain these assessment costs at a reasonable level, this sampling program was much smaller in scope than the original survey of the river conducted during 1993–1996.

How did the decision analysis described in this paper affect decisions made by the GLFC? As elaborated on below, there were three important consequences of our analysis. First, our explicit consideration of uncertainty altered the choice of management option made by the GLFC, although not for the reasons typically described for decision analysis. Second, the analysis allowed the GLFC to justify a compromise management option that reflects a balance of short- and long-term interests. Third, the analysis cemented support for ongoing assessment in the St. Marys River to inform future decisions.

Our results convinced Commissioners that the control options of enhanced trapping and SMRT not only had sufficient promise, but were likely key to long-term, cost-effective control of sea lampreys in the St. Marys River.

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Prior to this analysis, key Commissioners, GLFC staff, and even some authors of this paper were skeptical about the potential of trapping and SMRT. By explicitly accounting for uncertainty, especially concerning sea lamprey demographics, we were able to demonstrate that enhancement of these methods had a high probability of contributing substantially to future suppression of sea lampreys in the St. Marys River. In the absence of our modeling results, it is likely that the GLFC would not have funded enhanced trapping and SMRT, principally because of a qualitative impression of the risks associated with these methods.

The outcomes of this analysis differed from the commonly cited reason why decision analysis can alter decisions, namely that the highest-ranked decision changes when the analysis explicitly accounts for uncertainty. When we ran simulations with sea lamprey demographic uncertainty removed (i.e., using the maximum posterior density estimates of the demographic parameters), the rank order of management options did not change, so adding demographic uncertainty did not actually affect the conclusions of the decision analysis. Instead, the effect of conducting the decision analysis was to give decision makers more confidence in the relative merits of what they had previously judged to be a risky decision based on their deterministic analyses. We expect that this is a more common consequence of conducting a decision analysis than has been previously reported in the fisheries literature.

Of course, we did not capture all sources of uncertainty in our analysis. For example, we only considered one set of functional forms for the models we used. One might argue, for example, that a Beverton–Holt rather than a Ricker function might equally or better approximate the relationship between recruitment and stock size. However, because we allowed for distributions of model parameters, we did allow for a wide range of shapes for the stock–recruitment function, even though they shared a common functional form. We think our approach appropriately captured uncertainty in the steepness of the stock–recruitment function at low stock sizes and in the amount of density-independent variation in recruitment, which are critical drivers of the results. We cannot, however, rule out that some other aspect of our model could be treated in a plausible but different way than ours, which might have altered the results of the decision analysis. This emphasizes that decision analyses are almost always going to understate the true amount of uncertainty. A pragmatic approach is to focus on plausible alternative functions or models, where intuition and experience suggest that the different models might produce different results. If sensitivity analysis suggests the alternative form changes the decision analysis results, then one could consider incorporating this model uncertainty formally into the analysis.

Our results suggested that an option without any use of Bayluscide (option 5) performed nearly as well as a similar option that included application of Bayluscide to a small area (option 8). It could be argued that the better choice would have been to select the less expensive option 5 and use the savings to fund control in other streams. However, option 8 had slightly higher net economic benefits than option 5 and resulted in a more rapid decline of sea lamprey numbers in the early years of the simulations (prior to 2012, not shown here). Thus, option 8 allowed the Commissioners to hedge their bets on the payoff of nonchemical controls in the short term without suffering a reduction in expected net benefits over the long term. They decided that until enhanced alternative control has actually proven itself as an effective strategy, as evidenced by sustained reductions in sea lamprey recruitment to the St. Marys River, it would be prudent to continue using Bayluscide at some level.

Finally, the large uncertainty associated with the performance of all options convinced Commissioners to support an ongoing assessment program to detect strong recruitment events if they were to occur despite effective reductions in spawner abundance through trapping and SMRT. This program is valuable for three reasons. First, it yields new data on sea lamprey recruitment dynamics that can be used to reduce uncertainty about the stock–recruitment relationship and thus potentially contribute to better-informed decisions in the future. Second, it provides valuable data on spatial distributions of sea lampreys that can be used to guide application of future Bayluscide treatments. Most important, though, it provides the information required to implement a state-dependent control policy that will almost certainly be superior to the options considered in our decision analysis. Under such a policy, future Bayluscide treatments will not be fixed on a 4-year cycle, but rather will be triggered by observations of sea lamprey densities that are sufficient to warrant a chemical treatment. Both Haeseker et al. (2003) and Jones et al. (2003) have shown that sea lamprey populations can exhibit very large, density-independent variations in recruitment even when the spawning population is suppressed. A monitoring program with the ability to detect large recruitment events and a control option (Bayluscide) available to deal with these situations can greatly increase the efficiency of the control program compared with a program that simply relies on a fixed implementation schedule. A simple version of such a feedback rule is now being implemented in which St. Marys River plots are selected for Bayluscide treatments by comparing the abundance and size-structure of larvae observed in the plots with those observed in other streams being considered for lampricide treatment. In the future, we plan to revisit the decision analysis with a new model that allows evaluation of such state-dependent feedback policies.

The decision analysis described in this paper also has had broader consequences in the Great Lakes region. Interest in the explicit consideration of uncertainty for fishery management has grown considerably in the region since the start of this project. In parallel with this sea lamprey study, we also collaborated with Lake Michigan fishery managers to conduct a decision analysis for Chinook salmon hatchery stocking options in that lake (Szalai 2003). More recently, we have assisted Lake Erie fishery managers with a decision analysis for walleye (*Sander vitreus*) harvest management (Wright et al. 2005), and a similar analysis is underway for yellow perch (*Perca flavescens*) in Lake Michigan. Even the stakeholder community has begun to argue for more effective consideration of uncertainty in fishery management decisions (K. Reid, Ontario Commercial Fishery Association, 45 James Street, Blenheim, ON N0P 1A0, personal communication; S. Crawford, University of Guelph, Department of Integrative Biology, Guelph, ON N1G 2W1, personal communication).

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The key to success with practical, consequential applications of decision analysis lies in effective engagement of those involved in decision-making and managing the resource. The main reason the St. Marys River decision analysis has been influential is because the GLFC, the Sea Lamprey Integration Committee, and the agents for sea lamprey control (the US Fish and Wildlife Service and Fisheries and Oceans Canada) were actively involved in the decision analysis project from the start. Not only do decision makers, managers, and those who deliver the control program have an obvious and irreplaceable role in determining management objectives and options, but their involvement allows them to take ownership of the analysis. This may not be critical to ensure the analysis is technically sound, but without it the products of the analysis run a very high risk of being largely of academic value.

These successes have not come easily. Rosenberg and Restrepo (1994) stress the importance of estimating and communicating uncertainty effectively to fishery managers, who must weigh the benefits, costs, and risks of various management options. Those authors note that many managed fisheries have incorporated particular elements of uncertainty and risk analysis, but the advice resulting from these analyses has not always been expressed to decision makers in an effective manner. It is therefore incumbent upon scientists to work harder on this communication step.

Decision analysis and stochastic modeling are powerful tools for evaluation of fishery management issues. A large and steadily increasing body of literature describes these tools and then illustrates their merits when applied to carefully chosen examples (e.g., Punt and Hilborn 1997). Our paper adds to this literature, not so much by presenting new techniques for estimation or forecasting, but rather by illustrating an application of known techniques to a real, economically and ecologically important issue in fisheries management. We fear there is a larger gap than there should be between experts who are developing these techniques and decision makers who can benefit most from their use. We encourage both decision makers and experts to narrow this gap by engaging one another in the confrontation of real, "messy" problems and then telling the rest of us what happened.

Acknowledgements

Our analysis relied extensively on the data and expertise of the sea lamprey control and assessment staffs of the US Fish and Wildlife Service and Fisheries and Oceans Canada and also on the assistance of researchers from the US Geological Survey, Hammond Bay Biological Station. We thank the members of the Sea Lamprey Integration Committee and the GLFC for their active participation in this decision analysis. John Netto provided valuable assistance in programming the simulation model used to conduct the decision analysis. Funding for this study was provided by the Michigan Sea Grant College Program, the GLFC, and Michigan State University (MSU). This is publication 2007-05 of the Quantitative Fisheries Center at MSU. We appreciate valuable comments and suggestions from two anonymous reviewers.

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National Research Council Canada
Ottawa, Canada K1A 0R6

Conseil national de recherches Canada
Ottawa, Canada K1A 0R6

Postage paid at Ottawa
Publications mail
Registration No. 40062591

Port payé à Ottawa
Poste-publication
Enregistrement n° 40062591

USPS periodical postage paid at Plattsburgh, NY 12901, USA

Canadian Journal of Fisheries and Aquatic Sciences

Volume 64, Number 10, October 2007

Journal canadien des sciences halieutiques et aquatiques

Volume 64, numéro 10, octobre 2007

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Printed in Canada by / Imprimé au Canada par
NRC Reprographic Services / Service de reprographie CNRC
Page production by / Mise en page par
NRC Research Press / Presses scientifiques du CNRC

0706-652X(200710)64:10;1-Q